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Isomerization of Glucose to Fructose in Hydrolyzates from Lignocellulosic Biomass using Hydrotalcite

David Steinbach [1,*], Andreas Klier [1], Andrea Kruse [2], Jörg Sauer [1], Stefan Wild [1] and Marina Zanker [1]

[1] Karlsruhe Institute of Technology (KIT), Institute for Catalysis Research and Technology, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany; j.sauer@kit.edu
[2] University of Hohenheim, Institute of Agricultural Engineering, Garbenstrasse 9, 70599 Stuttgart, Germany; Andrea_Kruse@uni-hohenheim.de
* Correspondence: David.Steinbach@partner.kit.edu

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Abstract: The isomerization of glucose-containing hydrolyzates to fructose is a key step in the process from lignocellulosic biomass to the platform chemical hydroxymethylfurfural. We investigate the isomerization reaction of glucose to fructose in water catalyzed by hydrotalcite. Catalyst characterization is performed via IR, XRD and SEM. Firstly, glucose solutions at pH-neutral conditions are converted under variation of temperature, residence time and catalyst loading whereby a maximum of 25 wt.% fructose yield is obtained at 38 wt.% glucose conversion. Secondly, isomerization is performed at $pH = 2$ using glucose solutions as well as glucose-containing hydrolyzates from lignocellulosic biomass. Under acidic conditions, the hydrotalcite loses its activity for isomerization. In consequence, it is unavoidable to neutralize the acidic hydrolyzate before the isomerization step with an inexpensive base. As a neutralizing agent NaOH is preferred over $Ba(OH)_2$, since higher fructose yields are achieved with NaOH. Last, a pH-neutral hydrolyzate from lignocellulose is subjected to isomerization, yielding 16 wt.% fructose at 32 wt.% glucose conversion. This work targets the application of catalytic systems on real biomass-derived samples.

Keywords: glucose; fructose; aldose-ketose isomerization; pretreatment; hydrolyzate; lignocellulose; hydrotalcite; hydroxymethylfurfural; biorefinery; bioeconomy

1. Introduction

Producing platform chemicals from renewable lignocellulose, which is considered as a second generation biomass, has grown into a central point of interest. This is due to the limited availability of fossil resources, coupled with the aim to reduce the carbon footprint of such products [1]. One example is the production of hydroxymethylfurfural (HMF), which is one of the most important bio-based building blocks and can be converted into a variety of interesting chemicals [2]. HMF can be synthesized based on hexoses and their polymers, including lignocellulosic cellulose, which consists of glucose units. However, low yields from direct conversion of glucose to HMF in aqueous media are reported [2]. A previous isomerization of glucose to fructose could help to overcome this obstacle, because under the same reaction conditions HMF yields from fructose are higher than those from glucose [3].

The isomerization of glucose to fructose is carried out industrially with pH-neutral glucose solutions [4], which have a higher glucose concentration than process waters from the acid-catalyzed pretreatment of lignocellulose (hydrolyzates). In addition, the hydrolyzates are highly acidic and contain numerous by-products. Due to these different properties with regard to pH value, by-products and glucose concentration, a suitable isomerization catalyst for hydrolyzates has to be experimentally investigated.

Glucose cannot be completely converted to fructose in a single step, because the thermodynamic equilibrium ratio between glucose to fructose at 25 °C is 54:46 [5]. At higher temperatures the equilibrium shifts towards fructose, caused by the weak endothermicity of the reaction (standard reaction enthalpy of 2.8 kJ/mol [5]).

The conversion of glucose to fructose is an aldose-ketose isomerization, which can take place via two mechanistic pathways (see Figure 1). The first pathway is a keto-enol tautomerism, which is called Lobry-de-Bruyn-van-Ekenstein rearrangement. This rearrangement is catalyzed by Brønsted bases as a proton acceptor and takes place via the 1,2-endiol. In a second mechanistic scenario, a Lewis acid is used, which also abstracts a proton and an intramolecular hydrogen shift follows. This hydrogen shift was demonstrated in a study by Román-Leshkov and co-workers [6] using a Sn-β zeolite as a Lewis acid. This zeolite also catalyzes the ring opening of glucose into the acyclic form of the sugar [7]. Also, for the biochemical isomerization an intramolecular hydrogen shift takes place [8,9], so that the enzyme participates in the reaction in a similar way as a Lewis acid.

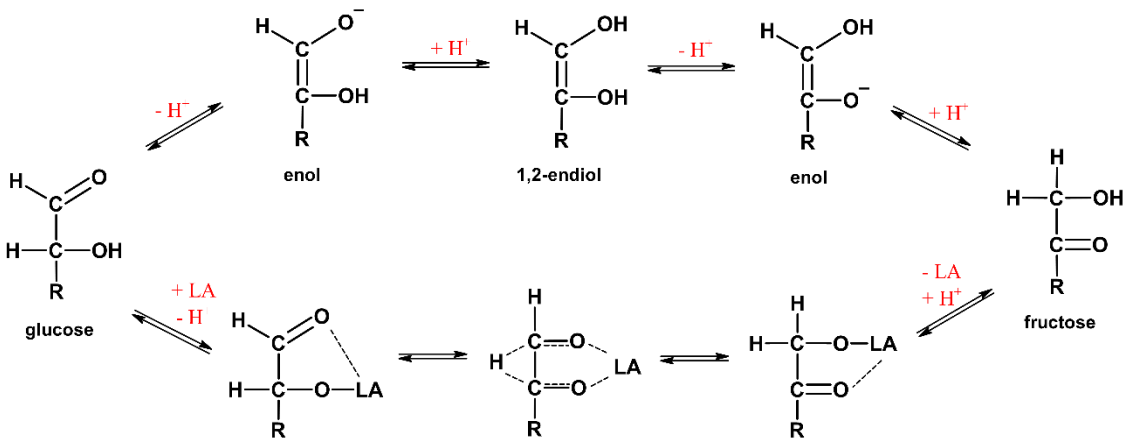


Figure 1. Mechanistic scenarios for isomerization of glucose to fructose [6], Brønsted-base-catalyzed keto-enol tautomerism with hydrogen transfer (top) and Lewis acid-catalyzed intramolecular hydrogen shift (bottom), LA: Lewis acid

Even without the presence of a catalyst, glucose isomerizes to fructose in hot high-pressure water [10]. However, in this case the isomerization is a side reaction with low fructose selectivities, whereas other reactions predominate, like glucose defragmentation. In order to accelerate the glucose isomerization, various homogeneous and heterogeneous catalysts were investigated, which are summarized by Delidovich and Palkovits [11]. In all studies, glucose is used as a pure substance in aqueous solutions. To the best of our knowledge, the isomerization of a hydrolyzate from the pretreatment of lignocellulose has not yet been investigated.

Heterogeneous catalysts are promising because they can be separated easily from the liquid phase for catalyst recycling. Both Brønsted bases and Lewis acids can be applied as a heterogeneous catalyst. Investigated heterogeneous Brønsted bases for isomerization include magnesium oxide, zirconium carbonate, hydrotalcite, attapulgite, zeolites modified with alkali and alkaline earth metals, microporous metal silicates and basic resins [11,12]. An example for Lewis acid heterogeneous catalysts are the group of Sn-β zeolites. The metal oxides of zirconium and titanium occupy an intermediate position, because they have both Lewis acid sites and base sites and thus act as bifunctional catalysts [13].

The isomerization results using hydrotalcites are summarized in Table 1. Hydrotalcites are magnesium-aluminum-hydroxycarbonates that occur as natural minerals or can be produced synthetically. The general formula is $[Mg_{1-x}Al_x(OH)_2]^{x+}[A]^{x-}\cdot mH_2O$, where x is the molar fraction of aluminum in the metal content and A symbolizes the anions, which are typically present as CO_3^{2-} and OH^-. Hydrotalcite forms layered structures with positively charged metal hydroxide layers and intermediate layers containing water and anions [14]. The anions in the interlayers are considered to be the active site for the isomerization [11]. The important aspect is, whether the anions are present

as CO_3^{2-} or OH^-, since OH^- has a higher basicity and is therefore more catalytically active than CO_3^{2-} [11,15]. In addition, the accessibility of the catalytic anions plays a role in the isomerization, since glucose cannot penetrate into the intermediate layers of the hydrotalcite [11,16].

By calcining the hydrotalcite, first water is released from the intermediate layers and at higher temperatures CO_2 from the carbonate anions [15]. This creates a mixed MgO-Al_2O_3 metal oxide with a large surface area [11,17]. However, the mixed metal oxides can incorporate anions into the intermediate layer, which again results in hydrotalcite, which is known as memory effect [15,17]. Rehydration with water causes OH^- anions, whereas CO_2 contact causes the formation of CO_3^{2-} anions in the intermediate layer [15]. The OH^- layer in the intermediate layer is not stable in the presence of air and is converted into the carbonate form by CO_2 absorption [16].

Yu et al. [17] investigated the influence of hydrotalcite calcination and rehydration on glucose isomerization. The calcined catalyst provided a high glucose conversion with poor fructose selectivity, which is caused by strongly basic sites of the mixed oxides [17]. On the other hand, the hydrotalcite rehydrated after calcination in water showed a high fructose selectivity, which is explained by weakly basic sites [17]. In a similar study by Delidovich and Palkovits [18], the hydrotalcite rehydrated in water gave the best fructose yields. A further work showed that targeted catalyst synthesis influences the basicity of the hydrotalcite [16]. A higher basicity not only leads to a higher glucose turnover, but also to a higher fructose yield [16]. Additionally, hydrotalcite can be regenerated by burning off organic deposits and rehydrating in water [16].

Table 1. Isomerization of glucose to fructose using hydrotalcite catalysts

temperature [°C]	reaction time [min]	glucose conversion [wt.%]	fructose selectivity [wt.%]	fructose yield [wt.%]	ref
90	60	28	78	22	[15]
90	120	41	75	31	[18]
110	180	34	89	30	[16]
95	?	42	60	25	[19]
120	60	43	64	27	[20]
100	?	18	78	14	[21]
90	120	61	83	50	[22]
100	300	27	74	20	[23]
100	300	61	70	42	[24]
100	180	38	66	25	[25]
120	240	36	75	27	[26]
100	300	54	56	30	[27]

No investigated heterogeneous catalyst enables a completely selective conversion of glucose into fructose. By-products are formed which differ in species and yield, depending on the applied reaction conditions and used catalyst. Most studies report sugar epimer mannose as a by-product. Using hydrotalcite as catalyst, reported by-products of isomerization are dihydroxyacetone, glycolaldehyde, lactic acid and traces of glyceraldehyde [18]. The two C3 compounds dihydroxyacetone and glyceraldehyde are formed by adol splitting of fructose [28]. Glyceraldehyde can only be found in traces because it dehydrates to methylglyoxal, which is further converted into lactic acid in a benzylic acid rearrangement [28,29]. The pH value of the product liquid drops due to the produced lactic acid, which leads to the removal of Mg^{2+} ions from the hydrotalcite [18].

In this study, we investigate the isomerization reaction of glucose to fructose in water, using hydrotalcite as a catalyst. Firstly, glucose solutions at pH-neutral conditions are converted under variation of temperature, mass ratio of catalyst to glucose and reaction time. Thereby, the optimal reaction conditions for glucose isomerization are determined. Catalyst characterization is performed via IR, XRD and SEM. Secondly, isomerization is performed at pH-acidic conditions, using glucose solutions as well as glucose-containing hydrolyzates from lignocellulosic biomass. Thereby, the catalytic performance at pH-acidic conditions is investigated. Last, a pH-neutral hydrolyzate is

subjected to isomerization, in order to compare isomerization of lignocellulose-derived sample with glucose solutions.

2. Materials and Methods

2.1. Obtaining a glucose-containing hydrolyzate from lignocellulosic biomass

An acid-catalyzed pretreatment of lignocellulose is performed to obtain a glucose-containing hydrolyzate. Beech wood chips (Joh. Sinnerbrink, Verl, Germany) or spruce wood chips (Hermann Keller, Achern, Germany) are used as educts. Diluted sulfuric acid is used as catalyst to hydrolyze hemicelluloses and cellulose polymers in lignocellulose structure to water-soluble sugar monomers.

A semi-continuous test rig is used for pretreatment, where the liquid phase is continuously exchanged [30]. This has the advantage that liberated water-soluble molecules like sugar monomers are removed from the hot reactor and thus largely protected from secondary reactions. 180 and 200 °C are used as reactor temperatures. The stainless-steel reactor has an internal volume of 100 ml and is loaded with 15 g pre-dried wood chips as a fixed bed before the experiment. Demineralized water or dilute acid solution is continuously feed into the reactor at a volume flow of 15 mL/min. Demineralized water is pumped through the reactor during the heating phase. When the target temperature in the reactor is reached, the feed stream is switched to 0.05 mol/L dilute sulfuric acid solution. The hydrolyzate leaves the reactor continuously and is cooled in a heat exchanger. Hydrolyzate sample is collected for about 1 hour acid-catalyzed lignocellulose hydrolysis and afterwards stored at 4 ° C until further processing. The main organic components in the hydrolyzate of spruce wood are 2.1 g/L glucose, 0.91 g/L hydroxymethylfurfural, 0.23 g/L furfural, 0.05 g/L levulinic acid, 0.03 g/L formic acid and 0.03 g/L acetic acid. Other sugars like mannose, xylose and fructose as well as short-chain organic compounds and high-molecular-weight substances such as humines are present in unknown quantities.

The hydrolyzates are neutralized to pH = 7 by adding a strong base. This is done with constant stirring and pH measurement using a glass electrode. Concentrated sodium hydroxide or barium hydroxide solutions are used as bases. After neutralization, the solution is filtered through a 0.45 µm nylon membrane filter.

2.2. Isomerization experiments

The hydrotalcite catalyst in powder form with the formula $Mg_6Al_2[(OH)_{16}|CO_3]\cdot 4H_2O$ was purchased (Sigma-Aldrich, St. Louis, MO, USA). The calcination was performed in a muffle furnace at 450 °C for 6 h with a continuous nitrogen purge. After the calcination, the catalyst is stored under nitrogen inert gas.

The isomerization is carried out in a glass apparatus. A defined amount of the calcinated catalyst and 40 ml of glucose solution or hydrolyzate are placed in a 100 ml three-neck bottom flask with a magnetic stirrer. On top of the flask, a reflux cooler is installed and the flask is heated in a preheated oil bath to the reaction temperature of 65 - 100 °C within about 20 min, whereby the reaction temperature is measured in the solution. The gas phase in the glass apparatus was either air or inert gas via continuously introducing nitrogen into a side neck. After the reaction time of 0-120 min has passed, the flask is removed from the oil bath and cooled by ambient air, while stirring continues. The content of the flask is then transferred quantitatively to a vacuum filtration unit and filtered through a 0.45 µm nylon membrane. 20 ml of deionized water is added to the vacuum filtration unit, to wash the filter cake.

When an error bar is shown in the following diagrams, the isomerization experiments were carried out at least in triplicate, otherwise as a single determination. Fructose yield after isomerization of hydrolysate is calculated based on glucose in hydrolyzate, not including other hexoses.

2.3. Analytics

Characterization of the liquid samples after isomerization was performed with several HPLC methods. Preliminary filtration with 0.45 µm GHP syringe filters (Pall, New York, NY, USA) was

performed to remove high-molecular-weight products. Glucose, fructose and mannose were separated at 35 °C in a Metrosep Carb 2 column (Metrohm, Filderstadt, Germany). An eluent with 0.1 mol/L sodium hydroxide and 0.01 mol/L sodium acetate was used with a flow rate of 0.5 mL/min. Sugars were quantified by an amperometric detector. As byproducts of the isomerization reaction, short-chain aldehydes and organic acids as well as furan compounds were considered. However, we could not detect those compounds in any sample. The furan compounds hydroxymethylfurfural, furfural and methylfurfural were separated at 20 °C in a Lichrospher 100 RP-18 column (Merck, Darmstadt, Germany) and quantified by a UV detector at 290 nm. Therefore, a water–acetonitrile eluent (9:1 v/v) at a flow rate of 1.4 mL/min was used. Short-chain aldehydes and organic acids like formic acid, acetic acid, lactic acid and levulinic acid were separated with an Aminex HPX-87H column (Biorad, Hercules, CA, USA) at a column temperature of 25 °C. The eluent was 0.004 mol/L sulfuric acid at a flow rate of 0.65 mL/min. Detection was performed by RI and DAD.

In the experimental runs at neutral pH-conditions, glucose and fructose were also quantified by an enzymatic test kit (Boehringer Mannheim/R Biopharm, Darmstadt, Germany). Quantification was performed on a spectrophotometer CADAS 200 (Dr. Lange, Berlin, Germany).

The concentration of organic carbon (TOC) in the liquid samples was determined using a Dimatoc 2000 (Dimatec Analysentechnik, Essen, Germany) applying the differential method. The sample is catalytically oxidized at 850 °C, whereby all carbon is converted into CO_2, which is measured with an IR detector. By adding phosphoric acid to the sample at 160 °C, only the inorganic carbon is converted to CO_2 and subsequently measured.

The infrared spectra of dried hydrotalcite catalyst were recorded with a FT-IR spectrometer 660-IR (Varian, Palo Alto, USA) in transmittance mode for wavenumbers of 4000 - 400 cm^{-1}. The IR spectroscopy was carried out using KBr pellets and obtained spectra were normalized.

The surface structures of dried catalyst were investigated via scanning electron microscopy (SEM) in a LEO 982 Gemini (Carl Zeiss, Jena, Germany) equipped with a Schottky-type thermal field emission cathode, secondary electron detectors (Everhart-Thornley, inlens), and a backscattered electron detector.

For elemental analysis, the hydrotalcite catalyst is completely solved with concentrated acids (HNO_3, HCl and HF in a volume ratio of 6:2:1). The microwave digestion takes place in a Mulitwave 3000 (Anton Paar, Graz, Austria), where the heating power is increased linearly to 500 W over 15 min. Then the temperature of 240 °C is maintained for 60 min. The analysis of the dissolved sample is carried out using optical emission spectrometry with inductively coupled plasma in the radial measurement mode on a 725 ICP-OES (Agilent, Santa Clara, CA, USA).

X-ray diffraction (XRD) provides information about the crystal structure of the dried catalyst. The measurement is carried out for 60 min on a X'Pert Pro (PANalytical, Almelo, Netherlands). A Bragg-Brentano arrangement with a copper anode and a nickel filter is used, the characteristic X-ray radiation K_α being used for the measurement.

3. Results and discussion

3.1. Glucose isomerization under pH-neutral conditions

The isomerization of glucose solutions with hydrotalcite has already been performed, for example by Lecomte et al. [15]. Under the same reaction conditions, similar fructose yields were obtained (see Figure 2). However, the glucose conversion in this work (34 wt.%) is higher than by Lecomte et al. [15] (27 wt.%), which mainly results from a different methodology for the calculation of the conversion. Lecomte et al. [15] subtracted out the glucose that was adsorbed on the catalyst (about 5 wt.%). Additionally, they identified mannose and psicose as by-products with a total yield of not more than 5 wt.% [15]. In contrast, no mannose was detected in this study.

We used a different hydrotalcite catalyst than Lecomte et al. [15]. The Mg/Al ratio has a strong effect on the catalytic properties for the isomerization reaction [24]. While the hydrotalcite used here has a Mg/Al ratio of 3, Lecomte et al. [15] used a catalyst with a Mg/Al ratio of 4.5. The basicity is highest at a Mg/Al ratio of 3 and decreases at lower as well as larger ratios [31]. Too high basicity can

lead to increased defragmentation reactions of the sugars to organic acids [17], which lowers the fructose selectivity. This was also shown by Moreau et al. [19], where a Mg/Al ratio of 3 led to a lower fructose selectivity than a Mg/Al ratio of 2.5.

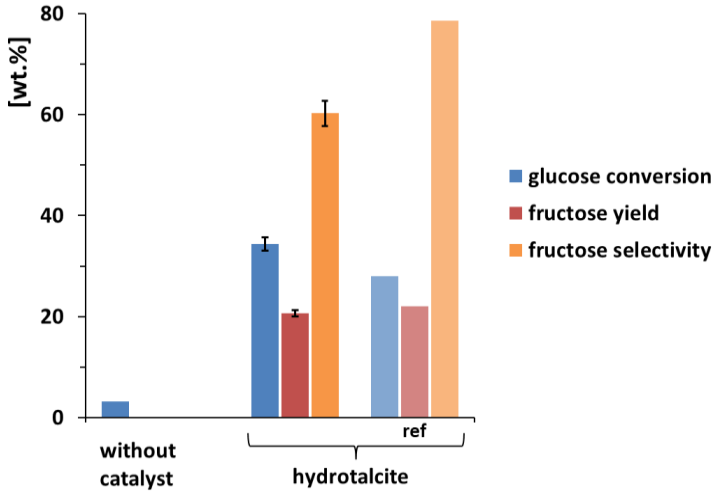


Figure 2. Glucose conversion and fructose yield using calcinated hydrotalcite, compared to Lecomte et al. [15] (ref), $c_{glucose}$ = 100 g/L, T = 90 °C, t = 60 min, air atmosphere, $m_{catalyst}/m_{glucose}$ = 0.2

Because the glucose concentrations after pretreatment of lignocellulose will be much lower than 100 g/L, applied in the study of Lecomte et al. [15], parameter studies in this work are carried out with diluted glucose solutions. The variation in the isomerization temperature in the range from 65 - 100 °C shows that, under mild reaction conditions of 65 °C, glucose can be selectively converted into fructose with hydrotalcite (see Figure 3 (a)). However, the yields are low (less than 10 wt.%). Higher temperatures lead to an increased glucose conversion, but the fructose selectivity decreases probably due to side reactions to unknown products, which lowers the fructose yield. However, no sugar defragmentation products (such as short-chain organic acids and aldehydes) or dehydration products (such as hydroxymethylfurfural and furfural) were detected via HPLC. Comparable studies with hydrotalcite found the sugar defragmentation products dihydroxyacetone, glycoladehyde and lactic acid [16,18]. However, the applied residence times of 1.5-48 h were much longer than in this work. The concentrations of the defragmentation products are probably below the detection limit due to the shorter reaction time.

The glucose conversion increases with longer reaction time (see Figure 3 (b)). The fructose yield has a maximum of 25 wt.% at about 60 minutes. With longer reaction times, the fructose yield decreases again. Hence the fructose formed could have reacted to secondary products. As a result, undesirable side reactions of both glucose and fructose occur. Subsequent reactions of fructose with longer residence times were also found by Souza et al. [32] during isomerization with NaOH as catalyst.

For the performed experiments, the mass ratio of catalyst to glucose has no influence on the fructose yield (see Figure 3 (c)). Thus, the number of active catalyst sites might not kinetically limit the isomerization reaction. The increase in the glucose conversion with a higher amount of catalyst suggests that glucose could deposit on the surface of the catalyst.

Whether the gas phase consists of air or nitrogen has no influence on the glucose conversion or the fructose yield (see Figure 3 (d)). Therefore, oxidation products with the ambient air play no role for the side reactions.

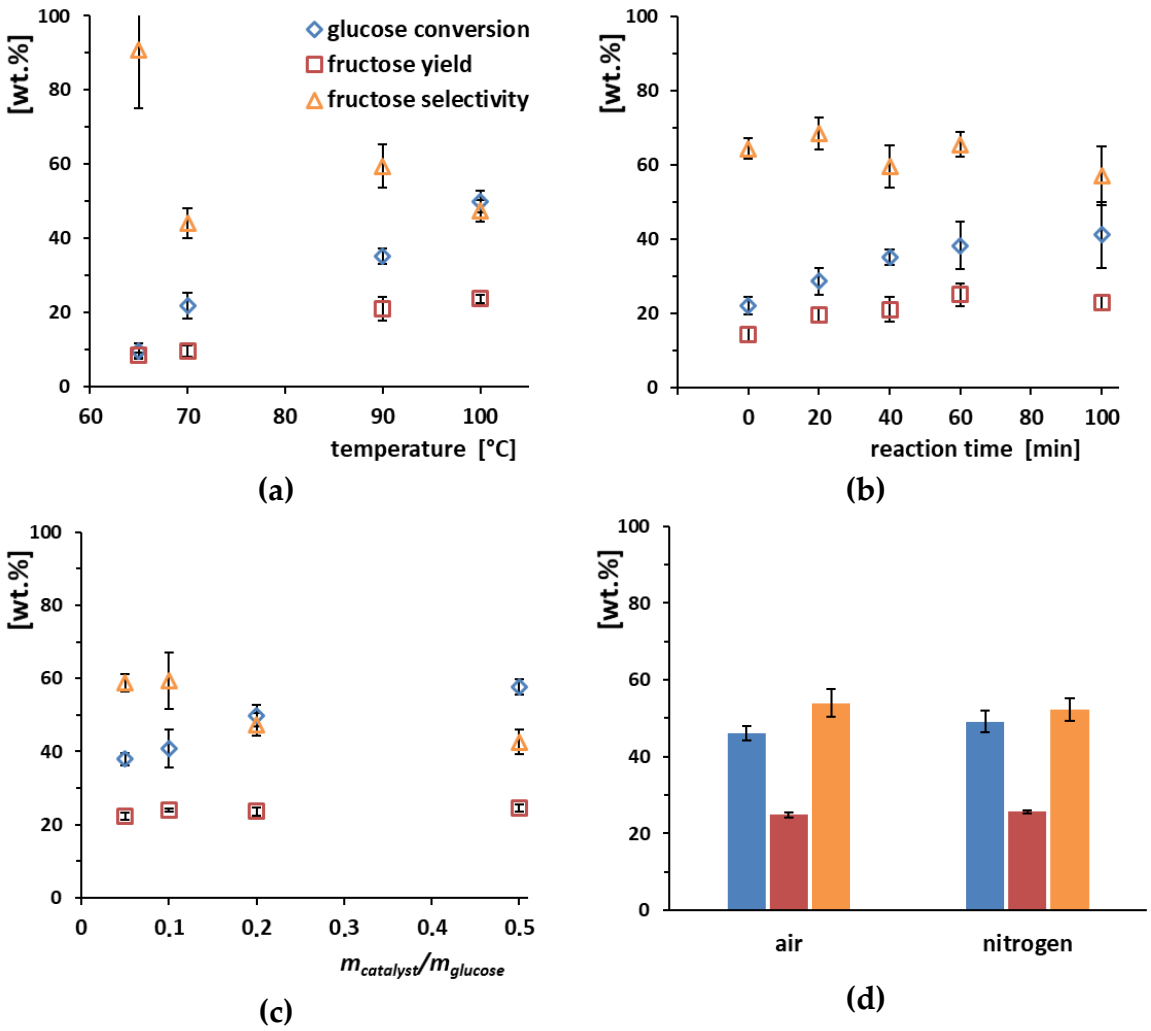


Figure 3. Glucose conversions and fructose yields using calcinated hydrotalcite with variation of (**a**) temperature, (**b**) reaction time, for $t = 0$ min sample is heated up to reaction temperature and then directly cooled down (**c**) mass ratio of catalyst to glucose at $T = 100$ °C and (**d**) gas atmosphere; always constant parameters: $c_{glucose} = 5$ g/L, $pH \approx 7$; parameters (unless otherwise stated): air atmosphere, $T = 90$ °C, $t = 40$ min, $m_{catalyst}/m_{glucose} = 0.2$

Because the glucose conversion is always much higher than the fructose yield, side reactions or deposition of glucose occur during isomerization. Figure 4 shows two carbon ratios to determine whether possible by-products are dissolved in the liquid phase. In the case of the reference point in Figure 4, no by-products are formed and the fructose selectivity is 100 wt.%. There are three model cases: (1) If the data point moves to the left in the diagram, by-products dissolved in water occur. (2) A shift of the data point on the bisector is caused by water-insoluble by-products. (3) If the data point is below the bisector, this is due to measurement errors. The experimental data from the isomerization cannot be clearly assigned to any of these three cases. However, most runs are closer to the bisector, which is associated with water-insoluble by-products. Either this could be deposits on the catalyst, or soluble polymers are formed under reaction conditions, which precipitate before sample analysis. The latter soluble polymers were reported by Souza et al. [32] as a by-product of isomerization. On the other hand, Souzanchia et al. [21] reported the formation of undesired insoluble by-products, mainly humins, on the surface of the used hydrotacite catalysts.

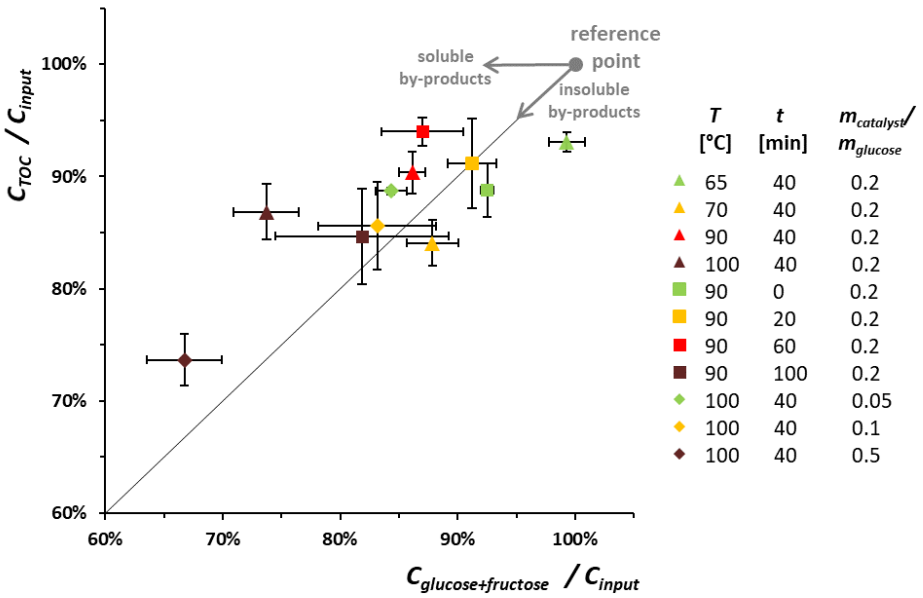


Figure 4. Carbon ratios for the isomerization of glucose with calcinated hydrotalcite; Y axis: TOC value of the product liquid to carbon input of the glucose solution C_{TOC}/C_{input}; X axis: carbon of glucose and fructose in the product liquid to carbon input of the glucose solution $C_{glucose+fructose}/C_{input}$; reaction conditions: $c_{glucose}$ =5 g/L, $pH \approx 7$, air atmosphere

The IR spectra of the catalyst before and after the isomerization show only slight differences (see Figure 5 (a)). Both spectra are dominated by a broad O-H stretching vibration at 3450 cm^{-1} [33,34]. The band at 1630 cm^{-1} is referred to O-H deformation vibrations [33]. The weak band at 760 cm^{-1} could be signals from Al-OH [33]. The bands in the range of 480 – 440 cm^{-1} are attributed to Mg-O-Al vibrations [33]. All other bands (1430, 1370, 760, 660 cm^{-1}) can originate from carbonates [29,33-35]. A precipitation of large amounts of organic matter during the isomerization on the catalyst surface is not detectable by IR spectroscopy, because no vibrations of organic compounds are found. For example, the spectra of the catalyst after isomerization show neither C-H valence vibrations at 2900 cm^{-1}, nor C=C valence vibrations of aromatics at 1600 - 1500 cm^{-1}.

XRD is also used to characterize the catalyst (see Figure 5 (b)). In the untreated hydrotalcite two sharp, symmetrical signals occur at 11.5° and 23.2° with high intensity. Weaker signals can be found at 34.8; 39.8; 46.7; 60.7 and 62.0°. The sharp XRD signals show that the untreated hydrotalcite catalyst has a crystalline structure and can be used to characterize the layered crystal structure [36,37]. The diffraction angles of the commercially available hydrotalcite used here are identical to other synthesized hydrotalcites [16,17,29,33].

After calcining the hydrotalcite, all XRD signals of the layered crystal structure have disappeared. So, the crystalline structure has been lost and disordered Mg-Al mixed oxides have formed [17,29,38]. After the isomerization, part of the crystalline order in the hydrotalcite returns, which can be seen from the XRD signals at low diffraction angles. This could be achieved by rehydration of the catalyst with water from the isomerization solution, whereby the layered crystal structure is restored by the "memory effect" [17,29,38].

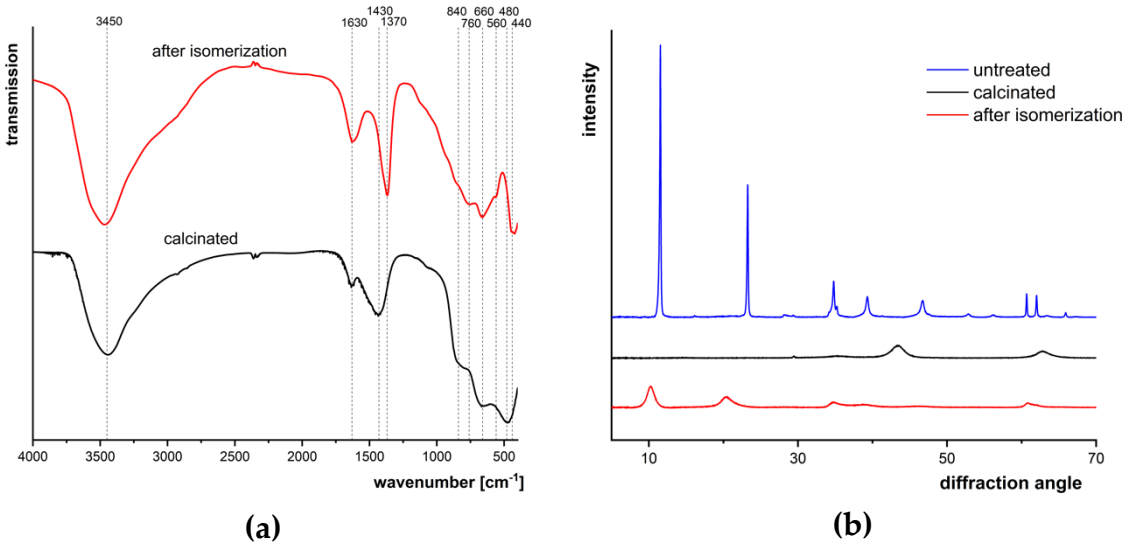


Figure 5. (**a**) IR spektra and (**b**) XRD pattern of hydrotalcite; reaction conditions: $c_{glucose}$ = 5 g/L, $pH \approx 7$, air atmosphere, T = 100 °C, t = 40 min, $m_{catalyst}/m_{glucose}$ = 0.2

3.2. Glucose isomerization under pH-acidic conditions

By now, isomerization with hydrotalcite has only been investigated under pH-neutral reaction conditions. However, the hydrolyzate from the acid-catalyzed hydrolysis of lignocellulose is produced with an acidic pH-value. Therefore, it is investigated here, whether the hydrotalcite catalyzes the isomerization even under acidic conditions. As can be seen in Figure 6, the glucose conversion drops sharply, if the pH value is decreased. This effect can be compensated by a larger mass ratio of catalyst to glucose.

In addition, the dissolved metal ions in the product liquid are analyzed. During the isomerization, especially magnesium ions are released from the catalyst. For example, 3.2 wt.% of the magnesium bound in the hydrotalcite goes into solution, in the run shown in Figure 6 with $m_{catalyst}/m_{glucose}$ = 0.5. In contrast, aluminum remains almost completely in the solid.

Under acidic pH conditions, hydrotalcite loses its activity for isomerization, which can be concluded from the decreased glucose conversion at pH = 2. The anions in the hydrotalcite solid structure are OH^- ions after calcination and subsequent suspension in water [15]. These hydroxides are neutralized with the hydronium ions of sulfuric acid. This also makes the metal cations partially water-soluble.

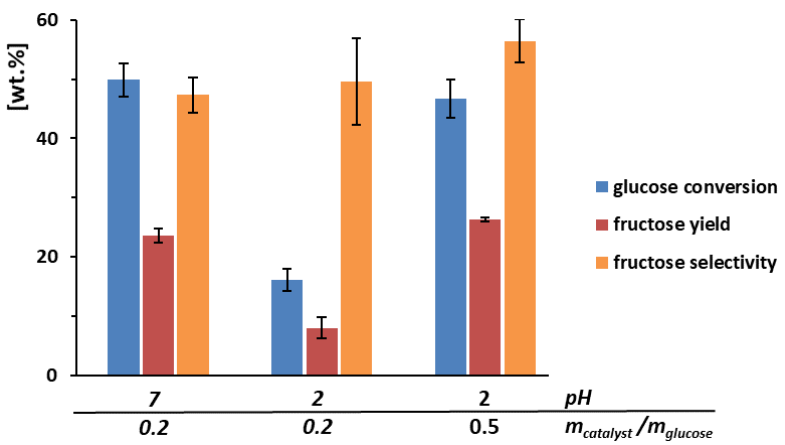
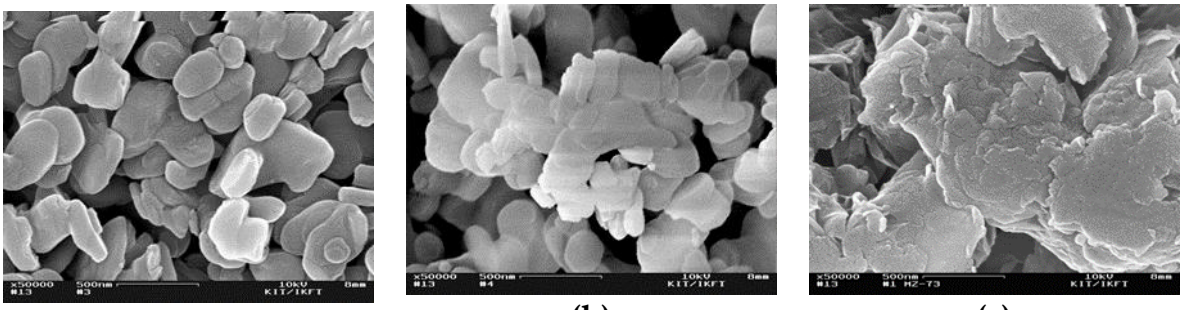


Figure 6. Glucose conversions and fructose yields with calcinated hydrotalcite with variation of the pH value by adding sulfuric acid, constant parameters $c_{glucose}$ = 5 g/L, air atmosphere, T = 100 °C, t = 40 min

After the isomerization under acidic pH conditions, a clear change in the catalyst morphology is visible (see Figure 7). The catalyst appears to have partially dissolved and re-agglomerated into new particles of different morphology. The particles after the isomerization reaction are much larger than the particles of the calcinated catalyst before the start of the reaction.



(a) **(b)** **(c)**

Figure 7. SEM images of the catalyst hydrotalcite in 50,000x (**a**) before calcination, (**b**) calcinated and (**c**) after isomerization; reaction conditions: $T = 100$ °C, $t = 40$ min, $pH = 2$, air atmosphere, $m_{catalyst}/m_{glucose} = 0.2$

3.3. Isomerization of hydrolyzate from lignocellulose without previous neutralization

If the acidic hydrolyzate is exposed to the typical reaction conditions (100 °C, 60 min) of the isomerization without the addition of hydrotalcite catalyst, no fructose is produced but almost 60 wt.% of the dissolved glucose is converted (see Figure 8). This shows that the organic compounds in the hydrolyzate are very reactive in a pH-acidic medium.

When hydrotalcite is added in a catalyst/glucose ratio of 0.1, no fructose is formed. In contrast, in the case of pH-neutral glucose solutions, this catalyst loading was sufficient for fructose formation (see Figure 3 (c)). Presumably, the catalytic active OH^- ions in the hydrotalcite structure are neutralized by the sulfuric acid from the hydrolyzate, as discussed in the previous chapter. The alkaline structure of the catalyst is lost and the isomerization can no longer be catalyzed. This neutralization effect of the catalyst can be compensated by offering hydrotalcite in excess. However, using the isomerization catalyst as a neutralizing agent is not useful. Rather, the hydrolyzate should be neutralized before the isomerization by an inexpensive base.

Fructose is formed at a mass ratio of catalyst to glucose of 1 (see Figure 8). Even if this ratio is increased further, the fructose yield remains relatively constant at around 11 - 15 wt.%. In general, these fructose yields of the hydrolyzate are lower than the results with glucose solutions. In addition, the glucose turnover in the hydrolyzate is much higher compared to a glucose solution.

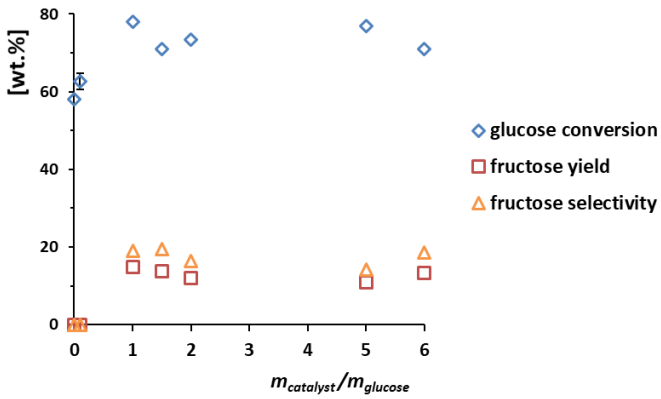


Figure 8. Glucose conversions and fructose yields of the calcinated hydrolyzate from lignocellulose with varying mass ratio of hydrotalcite catalyst to glucose; pretreatment parameters: beech wood,

T = 180 °C, 0.05 mol/L H_2SO_4; isomerization parameters: $c_{glucose}$ = 2.5 g/L, air atmosphere, T = 100 °C, t = 60 min, pH = 1.3

3.4. Isomerization of hydrolyzate from lignocellulose after previous neutralization

The two Brønsted bases NaOH and $Ba(OH)_2$ are used to neutralize the hydrolyzate from the sulfuric acid-catalyzed pretreatment of lignocellulose. When the pH is adjusted to 7, the hydrolyzate changes color from light brown to dark brown for both neutralizing agents. While the neutralization product Na_2SO_4 is highly water-soluble, $BaSO_4$ is almost insoluble (2.3 mg/L [39]). Therefore, a precipitate is formed in the hydrolyzate immediately after addition of $Ba(OH)_2$. This difference in solubility and its effect on the process was the reason to use $Ba(OH)_2$ as well as NaOH.

The organic compounds remain largely dissolved in both neutralization variants. The analysis of the carbon concentration before and after neutralization shows a recovery of 91.7 % for NaOH and 97.6 % for $Ba(OH)_2$. In addition, concentrations of measured byproducts from acid-catalyzed pretreatment of lignocellulose (hydroxymethylfurfural, furfural, levulinic acid, formic acid and acetic acid) were identical before and after neutralization step. The solid precipitate of the $Ba(OH)_2$ neutralization is examined via IR spectroscopy and shows agreement with the typical IR bands of $BaSO_4$, while typical bands of organic compounds are not dominant (data not shown). So, if a salt is precipitated by the neutralization, no larger amounts of organic matter adsorb on it or co-precipitate.

No measurable fructose yields can be achieved with neutralized hydrolyzate, when a hydrotalcite catalyzed isomerization at mild reaction conditions of 60 °C and 40 min is performed. However, fructose is obtained if the temperature is increased to 100 °C (see Figure 9). The mass ratio of catalyst to glucose in the examined range of 0.2 to 0.5 has no strong influence on the isomerization (see Figure 9 (b)). If isomerization takes place under inert gas, the fructose yield seems higher than with ambient air (see Figure 9 (c)). When the hydrolyzate is neutralized with NaOH before isomerization step, a much higher fructose yield can be achieved, compared to neutralization with $Ba(OH)_2$ (see Figure 9 (a)). Using NaOH, the formed sodium sulfate salts remain solved in the isomerization step, whereas barium sulfate precipitates and is therefore not present in the isomerization step. The presence either of the Na^+ ions or the sulfate ions in the hydrolyzate appear to have a positive effect on the isomerization, which should be addressed in further studies.

The concentrations of byproducts from acid-catalyzed pretreatment of lignocellulose changed during isomerization. For example, following concentration changes occurred using nitrogen atmosphere, NaOH neutralization and $m_{catalyst}/m_{glucose}$ = 0.21 with conditions listed in Figure 9: Hydroxymethylfurfural (910 mg/L decreased to 710 mg/L), furfural (230 mg/L decreased to 50 mg/L), levulinic acid (50 mg/L increased to 610 mg/L), formic acid (30 mg/L increased to 260 mg/L) and acetic acid (30 mg/L increased to 160 mg/L). Thus, considerable amounts of organic acids are formed during isomerization step of biomass-derived hydrolyzate, whereas hydroxymethxylfurfural and furfural could be partly converted or adsorb on the catalyst surface.

By neutralizing the hydrolyzate before the isomerization step, it was possible to reduce the ratio of glucose conversions to by-products drastically. Using a pH-acidic hydrolyzate, 60-80 wt.% of glucose are converted while maximal 15 wt.% fructose being formed (see Figure 8). On the other hand, after prior neutralization, the glucose conversion is reduced to 32 wt.% and a fructose yield of 16 wt.% is achieved (see Figure 9). The previous neutralization presumably prevents acid-catalyzed side reactions of the glucose, which take place during the isomerization step under acidic conditions (e.g. fragmentation reactions). When the just mentioned isomerization results of neutralized lignocellulose hydrolyzate are compared with glucose standard solutions, a higher fructose yield is obtained with glucose standard solution under the same reaction conditions (23 wt.% fructose yield at 41 wt.% glucose conversion, see Figure 3). Further studies should show, if these differences are due to deactivation of the catalyst using the neutralized hydrolyzate. For example, the role of (1) byproducts from acid-catalyzed pretreatment of lignocellulose (other sugars, furfural, hydroxymethylfurfural, organic acids, high-molecular-weight humins) as well as (2) salts from pretreatment catalyst and neutralization agent should be investigated. In addition, the recovery and

regeneration of hydrotalcite catalyst after isomerization of hydrolyzate from lignocellulose pretreatment should be addressed.

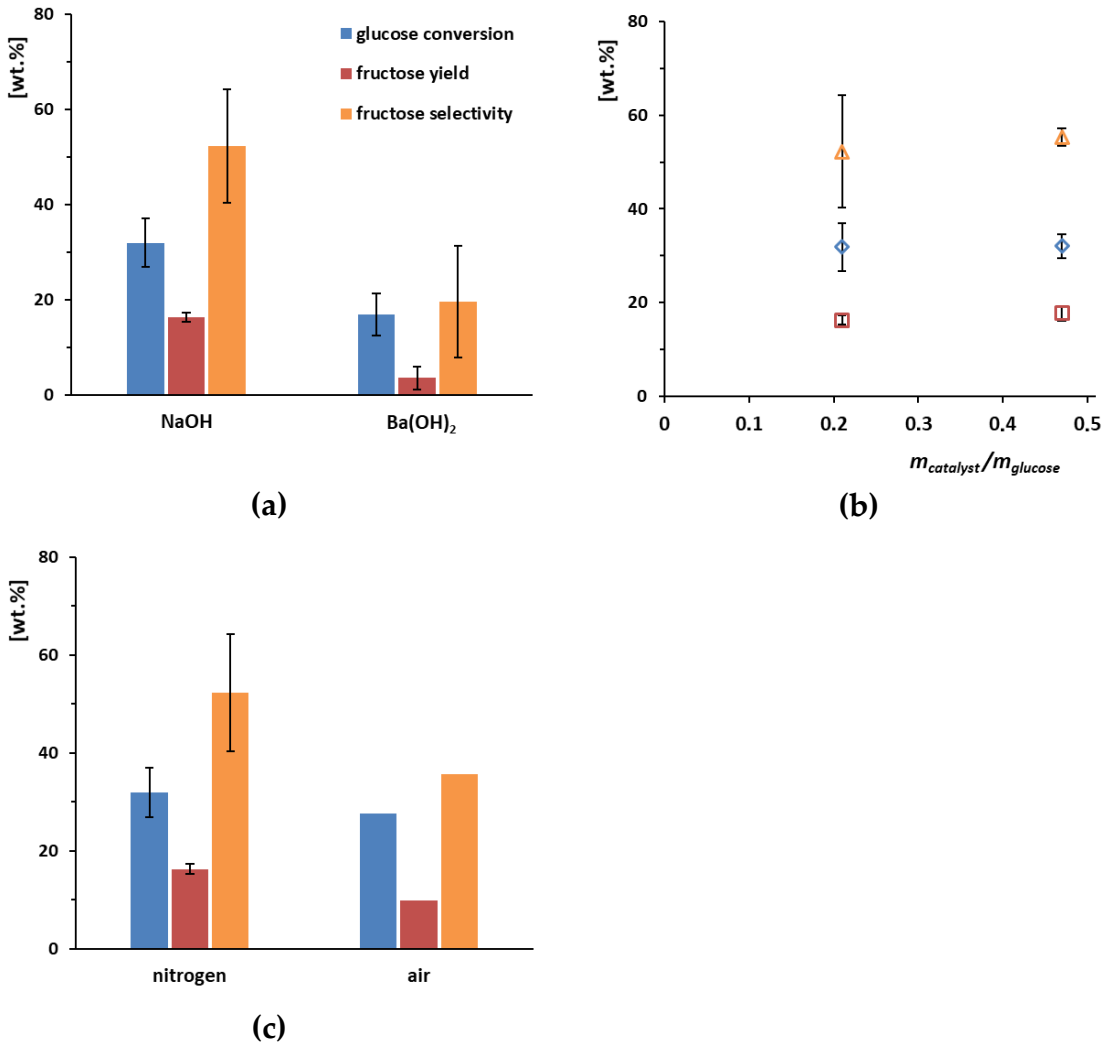


Figure 9. Glucose conversions and fructose yields of the neutralized hydrolyzate from lignocellulose with variation of (**a**) neutralizing agent, (**b**) mass ratio of calcinated hydrotalcite catalyst to glucose and (**c**) gas atmosphere; pretreatment parameters: spruce wood, T = 200 °C, 0.05 mol/L H_2SO_4; constant isomerization parameters: $c_{glucose}$ = 2.1 g/L, $pH \approx 7$, T = 100 °C, t = 40 min; parameters (unless otherwise stated): neutralization with NaOH, nitrogen atmosphere, $m_{catalyst}/m_{glucose}$ = 0.21

4. Conclusions

Parameter studies with glucose solutions under pH-neutral conditions show the best isomerization results (25 wt.% fructose yield, 38 wt.% glucose conversion) at a mass ratio of hydrotalcite to glucose of 0.2 at 90 °C for 60 min reaction time. Carbon balance suggests that side products are more likely water-insoluble.

Under acidic pH conditions, the hydrotalcite loses its activity for isomerization. The hydroxides in the solid structure are neutralized by hydronium ions in solution. Thereby the metal cations become partially water-soluble too and the morphology of the catalyst is altered. In consequence, it is unavoidable to neutralize the acidic hydrolyzate before the isomerization step with an inexpensive base. During neutralization, almost all organic compounds stay in the liquid state. As a neutralizing agent NaOH is preferred over $Ba(OH)_2$, since better fructose yields are achieved with NaOH (16 wt.% fructose yield, 32 wt.% glucose conversion).

The isomerization of glucose-containing hydrolyzates to fructose is a key step in the process from lignocellulosic biomass to the platform chemical HMF. The catalytic system should be better

understood, especially the occurring side reactions. If the yields of side products are known, the carbon balance can be improved. Besides studies of the catalytic system with pure model compounds, especially the application to real biomass-derived samples like hydrolyzates should be enhanced.

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