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EFFECT OF PROCESS CONDITIONS AND WEAK ACID CATION EXCHANGE RESIN ON ISOMERIZATION OF GLUCOSE

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Prosessiolosuhteiden ja WAC (weak acid cation) hartsin vaikutus glukoosin isomeroitumiseen
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Työn tarkoituksena oli tutkia tärkeimpien prosessiparametrien vaikutusta glukoosin isomeroitumiseen fruktoosiksi. Kirjallisuusselvityksen perusteella pH, viipymäaika ja lämpötila ovat tärkeitä tekijöitä, jotka vaikuttavat glukoosin reaktioihin. Kokeellisessa osassa tutkittiin näiden parametrien vaikutusta sekä puhtaalla glukoosiliuoksella että neljällä eri lignoselluloosasokeriliuoksella. Myös metallilisäyksen ja WAC hartsin katalysoivaa vaikutusta glukoosin isomeroitumiseen tutkittiin.

pH:n vaikutusta glukoosin isomeroitumiseen tutkittiin pH 6−12 välillä 60 °C lämpötilassa. Fruktoosia alkoi muodostua pH 10 tasolla. Fruktoosin osuus glukoosin ja fruktoosin kokonaismäärästä kasvoi mitä korkeampi lähtö pH oli. Fruktoosin osuus oli suurimmillaan 55−65 % pH:ssa 12. Fruktoosi saanto oli kuitenkin alhainen, koska suurin osa glukoosista ja fruktoosista hajosi edelleen orgaanisiksi hapoiksi ja muiksi hajoamistuotteiksi olosuhteissa, joissa fruktoosia muodostui. Fruktoosin muodostuminen hidastui merkittävästi tai pysähtyi kokonaan kuuden tunnin jälkeen, koska liuoksen pH oli laskenut lähelle neutraalia tai jopa alemmas muodostuneiden happoyhdisteiden vuoksi. Prosessilämpötilan vaikutusta glukoosin isomeroitumiseen tutkittiin 23−60 °C välillä. Korkeammissa lämpötiloissa isomeroitumisreaktio oli nopeampi ja fruktoosia muodostui enemmän. Fruktoosiosuus lähtö pH 11 näytteessä 6 tunnin jälkeen oli 10 % 23 °C:ssa, 33 % 40 °C:ssa ja 48 % 60 °C:ssa.

Kalsiumkloridin ja natriumkloridin lisäyksellä (0,2 mol/l) puhtaaseen glukoosiliuokseen ei ollut merkittävää vaikutusta glukoosin isomeroitumiseen fruktoosiksi tai hajoamiseen happoyhdisteiksi. Magnesiumkloridin lisäys katalysoi glukoosin isomeroitumista, mutta myös glukoosin ja fruktoosin hajoamista, jonka vuoksi myöskään magnesiumkloridia ei voida pitää tehokkaana katalyyttinä fruktoosin tuottamiseksi. WAC hartsi nosti glukoosiliuoksen pH:ta, jonka vuoksi fruktoosia muodostui enemmän, mutta tällöin saatiin alhainen fruktoosisaanto glukoosin ja fruktoosin hajoamisen seurauksena. Prosessiolosuhteiden ja WAC hartsin vaikutusta glukoosin isomeroitumiseen tutkittiin myös lignoselluloosasokeriliuoksilla. Glukoosiliuoksen puhtaudella ei ollut vaikutusta glukoosin isomeroitumiseen tai hajoamiseen.

ABSTRACT

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Effect of process conditions and weak acid cation exchange resin on isomerization of glucose
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93 pages, 44 figures, 12 tables and 3 appendices

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Keywords: glucose, fructose, isomerization, WAC resin, liquid-chromatography

The aim of this thesis was to investigate effect of the most important process parameters on glucose isomerization into fructose. Based on literature review, pH, reaction time and temperature can be named as one of the most important process parameters affecting reactions of glucose. In the experimental part, effect of these process parameters on glucose isomerization was investigated with using a pure glucose solution and four different lignocellulosic sugar solutions. In addition, catalytic effect of metal addition and WAC resin on glucose isomerization was investigated.

Effect of pH on glucose isomerization was investigated within pH range of 6−12 at 60 °C. Glucose began to isomerize into fructose at initial pH 10. Fructose fraction of the total amount of glucose and fructose increased with higher initial pH. Initial pH 12 led to the highest fructose fractions which were between 55−65 %. However, fructose yield was low because most of glucose and fructose degraded into organic acids and other degradation products. Fructose formation slowed significantly or stopped after 6 hours because pH had decreased close to neutral or even lower due to formation of acidic compounds. Effect of process temperature on glucose isomerization was investigated within 23−60 °C. Higher temperatures led to higher fructose fractions and isomerization reaction was faster under higher temperatures. The pure glucose solution which initial pH was 11 led to fructose fraction of 10 % at 23 °C, 33 % at 40 °C and 48 % at 60 °C after 6 hours.

Addition of calcium chloride and sodium chloride to the pure glucose solution for 0,2 mol/l did not affect significantly to the isomerization of glucose into fructose or degradation into acidic compounds. Magnesium chloride addition resulted higher fructose fractions but it cannot be considered as an effective catalyst for producing fructose since decrease of total concentration of glucose and fructose lead to low yields of fructose. WAC resin increased pH of glucose solution which led to higher fructose fractions but fructose yields were low due to glucose and fructose degradation. In addition, effect of process conditions and WAC resin was investigated with lignocellulosic sugar solutions. Purity of glucose solution had no effect on glucose isomerization or degradation.

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TABLE OF CONTENTS

1 INTRODUCTION 7
2 GLUCOSE CONVERSION INTO FRUCTOSE 8
2.1 Monosaccharides 9
2.1.1 Glucose 9
2.1.2 Fructose 10
2.2 Chemical catalysts 11
2.2.1 Base catalysts 12
2.2.2 By-product formation in alkaline conditions 31
2.2.3 Acid catalysts 33
2.3 Enzymatic catalyst 38
2.4 Summary 40
3 CHROMATOGRAPHIC SEPARATION OF FRUCTOSE FROM GLUCOSE 43
3.1 Production of concentrated high fructose corn syrup 43
3.2 Working principle 44
3.3 Resin type 45
3.3.1 SAC resin 46
3.3.2 WAC resin 48
EXPERIMENTAL PART 49
4 EXPERIMENTS 49
4.1 Materials and methods 49
4.2 Results 51
4.2.1 Effect of pH and reaction time on isomerization of glucose 51
4.2.2 Effect of temperature on isomerization of glucose 62
4.2.3 Catalytic effect of WAC resins on isomerization of glucose 65
4.2.4 Catalytic effect of metal addition on isomerization of glucose 73
4.2.5 Effect of pH adjustment over time on fructose formation 78
5 FINAL CONCLUSIONS 81
REFERENCES 85
APPENDICES 94

LIST OF ABBREVIATIONS

DMF	Dimethylformamide
GI	Glucose isomerase
GS	Glucose solution
HFCS	High fructose corn syrup
HMF	Hydroxymethylfurfural
HPAE-PAD	High performance anion exchange chromatography with pulsed amperometric detection
HPLC-PDA	High performance liquid chromatography photodiode array detection
ICP	Inductively coupled plasma
PEI	Polyethylenimine
SAC	Strong acid cation
SBA	Strong base anion
SMB	Simulated moving bed
WAC	Weak acid cation
WBA	Weak base anion

1 INTRODUCTION

Nowadays, production of high fructose corn syrup (HFCS) is an important industrial-scale process where some glucose is converted enzymatically into fructose utilizing glucose isomerase (GI) as a catalyst and then treated with liquid chromatography to get HFCS which have a higher content of fructose. (Khalilpour and Roostaazad, 2008; Viard and Lameloise 1992) Glucose can be converted into fructose also using chemical catalyst besides enzymes. Although, various chemical catalysts and process conditions have been investigated for hundreds of years, an effective chemo-catalytic system have not been discovered to date. (Delidovich and Palkovits, 2016; Lv et al., 2015)

Traditionally, in the production of HFCS, the chromatographic separation of glucose and fructose is carried out utilizing a strong acid cation (SAC) exchange resin in a calcium form (Viard and Lameloise 1992). There is not much data where other resin types than SAC is investigated to glucose treatment. However, Saari et al. (2010) have studied adsorption equilibria for several saccharides, such as glucose and fructose, on strong base anion (SBA) and weak base anion (WBA) and weak acid cation (WAC) exchange resins besides SAC exchange resin which have given some information how glucose react in the presence of other types of ion-exchange resins. Several experiments have been carried out to investigate also the ability of ion-exchange resins to act as a catalyst to the glucose isomerization reaction (Lv et al., 2015; Rendelman and Hodge, 1979; Langlois and Larson, 1956). Besides HFCS, glucose has many promising applications in fields of food, pharmaceutical, packaging and specialty chemicals industries in the future (Chatterjee et al., 2015; Marianou et al., 2016; Clark and Deswarte, 2015). It is important to understand how glucose react under different process conditions when investigating an effective chemo-catalytic system or even developing new promising applications utilizing glucose.

The aim of this thesis is to investigate effect of varying process conditions on glucose isomerization and degradation. Because not much research has done on investigating glucose treatment on other than SAC exchange resin, effect of WAC exchange resin on glucose isomerization and degradation is experimented. Literature part of this thesis includes review of glucose conversion into fructose with various chemical catalyst and also review of the enzymatic process of HFCS including enzymatic isomerization of glucose into fructose and chromatographic separation of fructose from glucose utilizing SAC exchange resin. In the

experimental part, effect of process conditions, such as reaction temperature, time and pH, on glucose isomerization and degradation are investigated. Also, catalyzing effect of metals, as calcium, magnesium and sodium, on isomerization and degradation reactions is studied. Lastly, experiments where glucose solution is carried out in presence of WAC exchange resin is investigated under varying process conditions. Five different glucose solutions in total are studied in the experimental part where four of them are lignocellulosic sugar solutions delivered from industry and one of them is only a pure glucose diluted into distilled water.

2 GLUCOSE CONVERSION INTO FRUCTOSE

Catalytic system that isomerizes glucose into fructose have been under the research for hundreds of years. Glucose can be converted into fructose either via chemical isomerization or enzymatic isomerization. Chemical catalysts can be further classified to base and acid catalysts. Isomerization through base catalysts was the first chemical catalyst that were found to catalyze the isomerization reaction of carbohydrates in 1885. (Delidovich and Palkovits, 2016; Lv et al., 2015) Nowadays, enzymatic glucose conversion into fructose is an industrial-scale process which uses glucose isomerase (GI) as a catalyst to the production of high fructose corn syrup (HFCS) (Souza et al., 2012; Marinaou et al., 2016; Parker et al., 2010). However, this industrial-scale enzymatic process to convert glucose into fructose have several drawbacks. For example, the isomerization reaction needs glucose which has a high purity and the isomerization reaction is efficient only within a very limited temperature and pH range. Additionally, the process needs a high amounts of glucose isomerase which is rather expensive. (Souza et al., 2012; Mushrif et al., 2014; Delidovich and Palkovits, 2016)

The limitations of the enzymatic isomerization have inspired researchers investigating to find an efficient and suitable chemical catalyst to convert glucose into fructose. Although a chemical catalyst for glucose isomerization into fructose have been under intensive research especially last decades, an efficient, environmental friendly and energy efficient catalytic system have not been discovered to date (Lv et al., 2015). A suitable chemical catalyst should give as good yields and selectivity towards fructose as possible without formation of a by-products and at the same time a process should be environmentally safe and non-toxic. In addition, the catalyst should be economically viable and easily available in large volumes.

Considering large-scale processes, a catalyst must be also easy to remove from the product. (Zhao et al., 2014)

At first, section 2.1 is going to introduce physical and chemical properties of glucose and fructose and a difference between these two monosaccharides. Then sections 2.2 and 2.3 are going to introduce reaction kinetics and several experimental results considering both a chemical as well as an enzymatic glucose conversion into fructose. However, the main focus in this thesis is on chemical isomerization.

2.1 Monosaccharides

Monosaccharides or sometimes called simple sugars is a generic term that includes compounds with the chemical formula of $(CH_2O)_n$, where n can vary between 4 and 9. (Schermann, 2008) Monosaccharides are further classified to aldoses and ketoses based on their most highly oxidized functional group. For example, if the highest oxidized functional group is aldehyde they are classified to aldoses and similarly if the highest oxidized functional group is a ketone they are called ketoses. (Ouellette and Rawn, 2015; Rabinowitz, 2009) Monosaccharides can combine with each other's through glycosidic bonds and form larger carbohydrates. Carbohydrates consisting of around 2−10 monosaccharides are called oligosaccharides. Carbohydrates which consists of thousands of covalently linked monosaccharides are called polysaccharides. One example of polysaccharide is cellulose. (Schermann, 2008; Rabinowitz, 2009; Ouellette and Rawn, 2015) The most common and important monosaccharides are the isomers glucose and fructose, which have the same molecular formula of $C_6H_{12}O_6$ (Murray, 1977; Schermann, 2008).

2.1.1 Glucose

Glucose, also known as dextrose, has the chemical formula of $C_6H_{12}O_6$, containing six carbon atoms and an aldehyde group which makes it an aldohexose (Murray, 1977; Dzoyem, 2014). Glucose is a building block of various polysaccharides, such as cellulose, starch, and glycogen. An enzymatic hydrolysis of corn is nowadays a common industrial process to produce glucose. Also, converting glucose from less expensive materials as wood and agricultural waste have been under investigation. (Chatterjee et al., 2015)

Glucose has many kinds of applications in fields of food, pharmaceutical, packaging and specialty chemicals industries (Clark and Deswarte, 2015). Glucose has been investigated as a renewable option to petroleum-based feedstocks for the manufacture of fuel components biochemicals and biomaterials. For example, glucose can be converted to ethanol, butanol, and lactic acid by fermentation, or to hydroxymethylfurfural (HMF) and levulinic acid by dehydration and decomposition. (Chatterjee et al., 2015; Marianou et al., 2016)

2.1.2 Fructose

Fructose has the same chemical formula of $C_6H_{12}O_6$ as glucose but have a different chemical structure, i.e. fructose and glucose are isomers of each other. Figure 1 presents a structure of glucose and fructose in a cyclic form (Watanabe et al., 2005). Fructose molecule contain ketone group and therefore called a ketohexose. (Marianou, 2016)

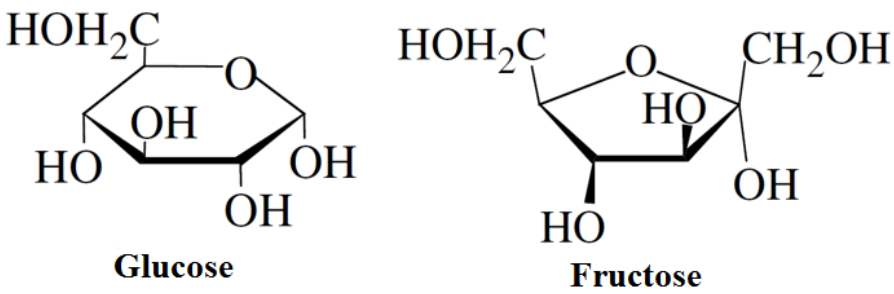

Figure 1 The structure of glucose and fructose molecule in the cyclic form (Adapted from Watanabe et al., 2005).

Important properties of glucose and fructose are summarized in Table I. Fructose is 2,35 times sweeter than glucose and has actually the highest sweetness from all the carbohydrates which occur naturally (Napari, 2007). Molar mass and volume are the same for glucose and fructose. However, fructose is more easily soluble in water than glucose. (Marianou, 2016; Pangborn, 1963; Wolfsdorf and Garvey, 2016; Johnson and Conforti, 2003)

Table I Properties of glucose and fructose.

	Glucose	**Fructose**	**Ref.**
Molar mass, [g/mol]	180.16	180.16	(Yalkowsky and He, 2003)
Van de Waals molar volume, [cm^3/mol]	88.03	88.03	(Telis et al., 2007)
Melting point, [°C]	146	129	(Yalkowsky and He, 2003)
Solubility[A], [g/l]	479	778	(Yalkowsky and He, 2003)
Viscosity[B], [mPas]	3.01	2.73	(Telis et al., 2007)
Relative sweetness compared with sucrose	0.74	1.74	(Napari, 2007)

[A] solubility in water at 20 °C
[B] concentration of monosaccharide 30 w-% in a temperature of 20 °C

Fructose occurs naturally in berries, honey, fruits, and can make even up to 5–8 % of their weight and honey consist of about 40 % of fructose. In addition, fructose is a building block of a sucrose which is also called table sugar. (Johnson and Conforti, 2003; Ziesenitz, 2015) Fructose is also a major component of a variety of syrups such as a high fructose corn syrups (HFCS). Syrups have a varying content of fructose ranging from 45–55 % which depend on the type of syrup. (Ziesenitz, 2015) Fructose is also a commonly utilized sweetener in food and beverage industries, due it has such important properties as sweetness, solubility, flavor enhancement, freezing-point depression and an excellent humectant (Pangborn, 1963; Wolfsdorf and Garvey, 2016; Johnson and Conforti, 2003; Hanover and White, 1993).

2.2 Chemical catalysts

Chemical isomerization of glucose into fructose is possible to perform either under basic or acidic conditions. A reaction mechanism of glucose isomerization is sometimes called “proton transfer” mechanism in the case of base catalysts and “intramolecular hydride shift” mechanism in the case of acid catalysts. (Marinaou et al., 2016) These reaction mechanisms are introduced in more detail at the beginning of sections 2.2.1 and 2.2.2. Although a great research interest has been shown towards both base and acid catalyst, here the main focus is more on introducing base catalyst due to nature and reaction conditions of investigated catalytic system of this thesis.

2.2.1 Base catalysts

Bases were the first chemical catalysts that was found to isomerize glucose into fructose as early as in 1885. This base-catalyzed glucose isomerization is also sometimes called as the Lobry de Bruyn–Alberda van Ekenstein transformation, which refers to the discoverers of this catalytic system. Figure 2 presents glucose molecule in a cyclic and an acyclic form where carbon atoms are named from one to six. (Mushrif et al., 2012) From now on, carbon (C) and oxygen (O) atoms are called by their numbers introduced in Figure 2, for example C1 present carbon atom which number is 1 and O1 present oxygen atom attached to carbon number 1 and so on.

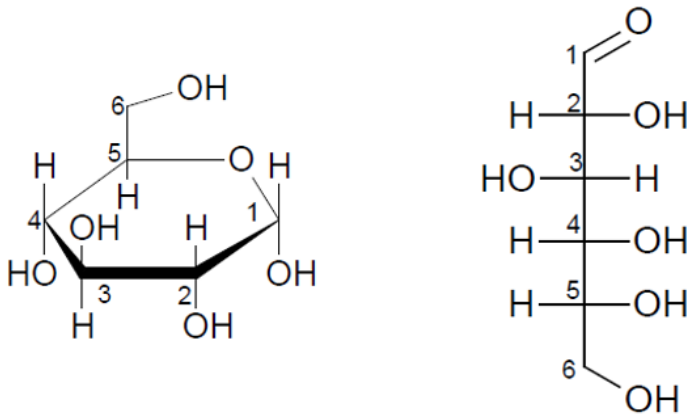

Figure 2 Cyclic (left) and acyclic (right) form of glucose molecule. Carbon atoms are named from 1 to 6. (Mushrif et al., 2012)

Carraher et al. (2015) have investigated the reaction mechanism of glucose conversion into fructose under basic conditions. The proposed mechanism includes reactions which causes the opening of the cycle, hydrogen transferring and closing the cycle. At first, H^+ is removed from OH-group of C1 which results to a glucose anion in the acyclic form. At this moment the charge is then in O5, which attracts to hydrogen atom to separate from C2. This leads to the formation of an enediol intermediate. The last step includes a formation of an acyclic fructose anion, closing the cycle and separation of a proton from water after which the final structure of fructose has been achieved. Schematic presentation of this base catalyzed glucose conversion to fructose is presented in Figure 3. (Marinaou et al., 2016; Carraher et al. 2015)

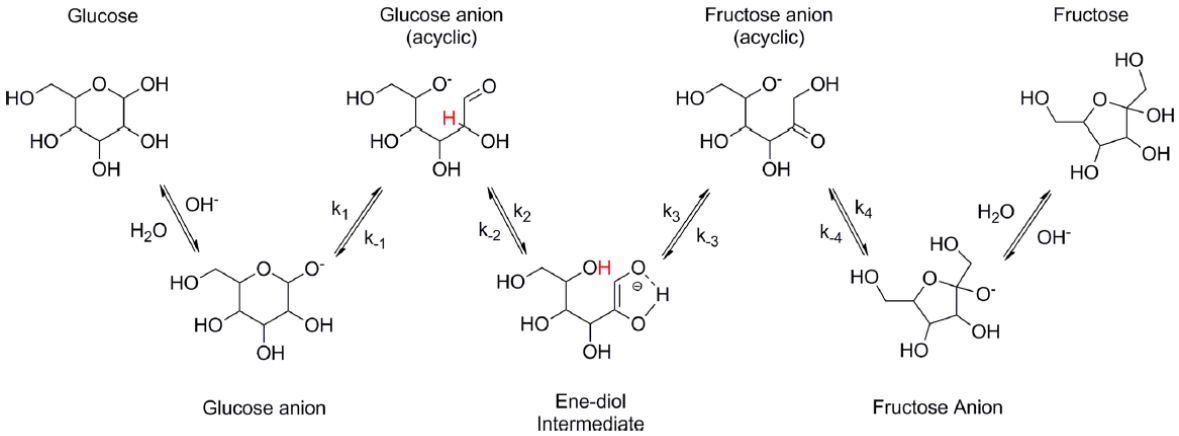

Figure 3 The reaction mechanism of the isomerization of glucose into fructose under basic conditions follows so called proton transfer mechanism (Carraher et al. 2015).

Soluble base catalysts

Early investigations of bases as a catalyst to glucose isomerization into fructose mainly focused on the research of soluble bases. For example, Bruijn et al. (1987) studied glucose conversion in water with potassium hydroxide (KOH) as a catalyst. The experiments were carried out at a temperature of 78 °C where glucose concentration was 0,45 wt-% and glucose/catalyst mass ratio was 8. The highest fructose selectivity of 61 % and yield of 11 % was observed in these reaction conditions. In addition, Delidovich and Palkovits (2014) have studied the promotion effect of NaOH catalyst on glucose isomerization into fructose and water as solvent at a temperature of 90 °C, where glucose concentration was 10 wt-% and a solution pH of 7,5 was adjusted with the addition of 1 mol/l NaOH. These reaction conditions obtained fructose selectivity of 70 % and 14 % yield at highest. (Delidovich and Palkovits, 2014)

Soluble bases as a catalyst seemed to result a low yield of fructose (10−15 %). However, Mendicino, (1960), Barker et al., (1975) and Bruijn et al. (1987) have done experiments where the yield of fructose is significantly increased by adding e.g. borates or aluminates. Mendicino (1960) performed experiments of glucose conversion into fructose in the presence of borates and sodium hydroxide in water. NaOH+$Na_2B_4O_7$ was used as a catalyst at a temperature of 100 °C, glucose concentration of 0,018 wt-% and glucose/catalyst mass ratio of 0,01 which resulted 85 % yield of fructose at highest. In addition, Barker et al. (1975) carried out experiments using NaOH+*p*-tolyl boric acid catalyst to glucose-fructose isomerization in water at a temperature of 50 °C where glucose concentration was 23,4 wt-%.

These reaction conditions gave 56 % fructose yield at highest. More recently, Despax et al. (2013) studied the effect of sodium aluminate ($NaAlO_2$) as a catalyst for glucose conversion into fructose and water as a solvent at a temperature of 55 °C where glucose concentration was 40 wt-% and glucose/catalyst mass ratio was 3,1. These reaction conditions resulted fructose yields of 41 % and selectivity of 70 % at highest. (Despax et al., 2013)

These improved yields of fructose are due to fructose forming a complex with an anion as borate or aluminate (Bruijn et al. 1987; Rendelman and Hodge, 1979; Delidovich and Palkovits, 2016). Kinetic studies carried out by Rendelman and Hodge (1979) have shown that hydroxide ion alone is responsible for glucose conversion into fructose, and that the aim of the aluminate ion is only to form complexes with fructose. Formation of a complex between fructose and aluminate causes increasing stability of fructose under basic conditions. (Rendelman and Hodge, 1979) Overall, borate and aluminate ions form more stable complexes with fructose than with glucose. Fructose forming complex with borate decreases the glucose concentration, which leads to glucose–fructose reaction equilibria to shift towards fructose. (Delidovich and Palkovits, 2016; Martin and Lichtenthaler, 2006)

Bruijn et al. (1987) have studied reaction rate constants for the isomerization and degradation of monosaccharides under alkaline conditions. Investigated monosaccharides were glucose, fructose, mannose and psicose. A simplified kinetic model shown in Figure 4 was used throughout in kinetic considerations. The reaction rate constants from k_1 to k_8 represent isomerization reactions and rate constants from k_9 to k_{12} represent degradation reaction of monosaccharides to acidic products. (Bruijn et al., 1987)

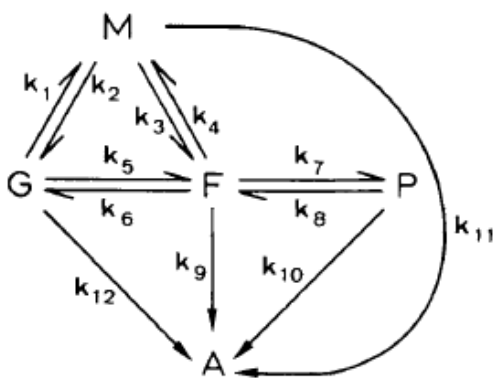

Figure 4 The simplified kinetic model for isomerization and degradation of monosaccharides under alkaline conditions, where G=glucose, M=mannose, F=fructose and P=psicose and A=acidic products and $k_{1\text{-}12}$ present reaction rate constants (Bruijn et al., 1987).

Effect of a hydroxide ion concentration on the reaction rate constants of isomerization and degradation reactions of monosaccharides is shown in Table II. Experiments were performed at a temperature of 78 °C were hydroxide ion concentration was increased with addition of potassium hydroxide (KOH) and concentration of monosaccharide was 0,025 mol/l. It can be seen that higher hydroxide ion concentrations promotes clearly isomerization reactions. For example, reaction rate constant of glucose isomerization to fructose (k_5) is increased from 20 to 360 with HO^- addition of 0,02 to 1 mol/l. (Bruijn et al., 1987)

Table II Effect of the hydroxide ion concentration on the (k_{1-8}) isomerization and degradation (k_{9-12}) reaction rate constants based to the kinetic model shown in Figure 4. Reaction conditions were 0,025 mol/l of monosaccharide and 78 °C. (Bruijn et al., 1987)

Rate constant (10^{-3} min^{-1})	HO^- concentration (M)				
	10^{-3}	$3.5 \cdot 10^{-3}$	10^{-2}	$3.5 \cdot 10^{-2}$	10^{-1}
k_1	1.1	3.0	7	15	25
k_2	2.8	8.0	18	40	60
k_3	8.8	26.0	49	115	160
k_4	2.7	8.0	15	35	60
k_5	20.0	55.0	115	260	360
k_6	15.7	45.0	90	220	330
k_7	4.2	12.0	25	60	90
k_8	8.0	23.0	45	100	140
k_9	7.9	24.0	50	135	220
k_{10}	12.0	36.0	70	190	320
k_{11}	1.1	3.0	7	19	31
k_{12}	1.6	5.0	10	27	46

Generally, divalent ions such as calcium ions in comparison with monovalent cations such as potassium or sodium ions, is known to promote degradation of monosaccharides under alkaline conditions. Kinetic experiments with calcium ion were carried out at a temperature of 78 °C where concentration of monosaccharide and potassium hydroxide was 0,025 mol/l and 0,01 mol/l, respectively. Calcium ion concentration was increased to 0,06 mol/l with an addition of calcium chloride. The impact of calcium ions on the isomerization and degradation is presented in Table III. It can be concluded that calcium promotes highly the formation of acidic products. Reaction rate constant (k_5) of glucose isomerization into fructose is increased from 115 to 120 with addition of 0,06 mol/l of $CaCl_2$. A formation of complexes between calcium ion and monosaccharide is responsible for the increasing degradation rates. Generally, the increase in the degradation rate (k_{9-12}) of monosaccharides after calcium addition is about five times higher than the increase of its isomerization

reaction rate (k_{1-8}). The increase in reaction rate constant is not that high in the case of isomerization but it still favors fructose formation. (Bruijn et al., 1987)

Table III Effect of calcium ion concentration on the (k_{1-8}) isomerization and degradation (k_{9-12}) reaction rate constants based to the kinetic model shown in Figure 4. Reaction conditions were 0,025 mol/l of monosaccharide, 0,01 mol/l of KOH and 78 °C. (Bruijn et al., 1987)

Rate constant (10^{-3} min^{-1})		Without Ca(II)	0.06 M $CaCl_2$
k_1	G → M	7	7
k_2	M → G	18	19
k_3	M → F	49	52
k_4	F → M	15	18
k_5	G → F	115	120
k_6	F → G	90	100
k_7	F → P	25	30
k_8	P → F	45	50
k_9	F → A	50	90
k_{10}	P → A	70	110
k_{11}	M → A	7	9
k_{12}	G → A	10	13

In addition, effect of borate addition on isomerization and degradation of monosaccharides was investigated. Table IV shows the results of borate addition and its effect to rate constants. Experiments were performed at a temperature of 78 °C were concentration of monosaccharide and potassium hydroxide was 0,025 mol/l and 0,01 mol/l, respectively. Potassium hydroxide concentration was reached with an addition of $Na_2B_40_7 \cdot 10H_2O$. Addition of borate to the monosaccharide solution seems to catalyze especially reaction towards ketose. This means that borate shifts the isomerization equilibrium towards fructose and psicose. For example, reaction rate constant (k_5) of glucose isomerization into fructose is increased from 115 to 120 with 0,4 borate to monosaccharide ratio but fructose isomerization into glucose is decreased from 90 to 53 with this same conditions. (Bruijn et al., 1987)

Table IV Effect of borate on the (k_{1-8}) isomerization and degradation (k_{9-12}) reaction rate constants based to the kinetic model shown in Figure 4. Reaction conditions were 0,025 mol/l of monosaccharide, 0,01 mol/l of potassium hydroxide at a temperature of 78 °C. (Bruijn et al., 1987)

Rate constant (10^{-3} min^{-1})		Molar borate/monosaccharide ratio		
		0	0.4	4
k_1	G → M	7	7	3
k_2	M → G	18	18	7
k_3	M → F	49	48	17
k_4	F → M	15	10	1
k_5	G → F	115	120	44
k_6	F → G	90	53	8
k_7	F → P	25	16	2
k_8	P → F	45	13	4
k_9	F → A	50	26	8
k_{10}	P → A	70	19	5
k_{11}	M → A	7	7	3
k_{12}	G → A	10	11	4

According to Bruijn et al. (1987), the presence of borate in alkaline solution with glucose and fructose leads to esterification of glucose and fructose with two borates forming 5-membered rings which is shown in Figure 5. Since borate forms esters with glucose and fructose under alkaline conditions. Differences between stabilities of esterified forms of glucose and fructose with borates are leading to the shift in the isomerization equilibrium. (Bruijn et al., 1987)

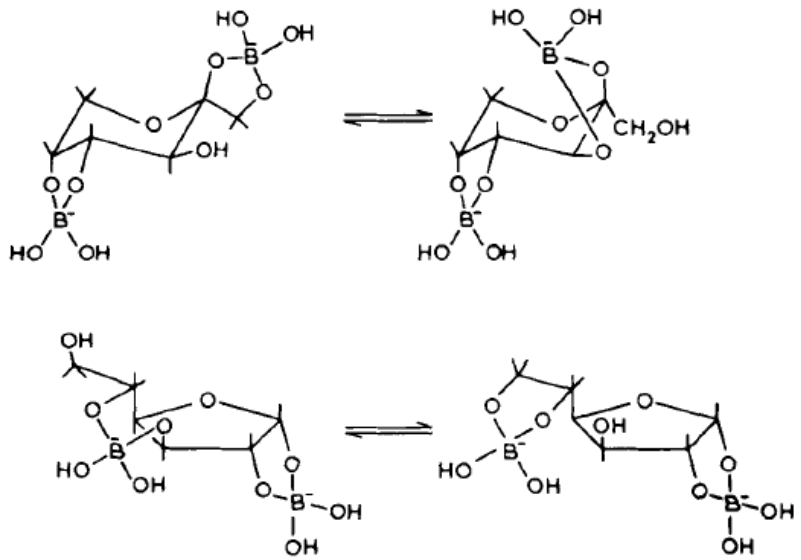

Figure 5 Schematic presentation of diborate ester forms of fructose (top) and glucose (bottom) (Bruijn et al., 1987).

Also, amines have been investigated as a soluble base catalyst to glucose conversion into fructose in water solution. It is suggested that the function of amines is only to generate hydroxide anions with a reaction with water. Figure 6 shows schematic presentation of hydroxide anion production where amine takes hydrogen ion from water to form hydroxide ions. Isomerization reaction takes place as the same way as shown earlier in Figure 3 after hydroxide generation. This means that solely hydroxide ions are responsible of the catalytic activity of isomerization reaction when using amines as a catalyst. Consequently, amines can catalyse glucose into fructose in water via similar reaction mechanism as earlier introduced soluble alkalis as sodium hydroxide or potassium hydroxide. (Carraher et al., 2015; Yang and Runge, 2016)

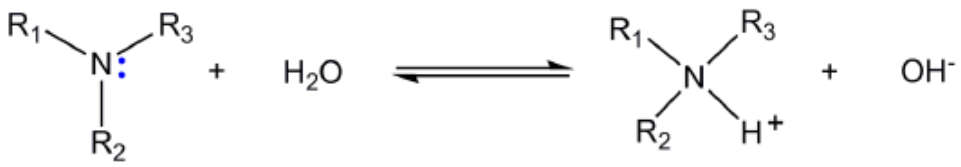

Figure 6 Reaction mechanism between amine and water to produce hydroxide anions (Yang and Runge, 2016).

In early investigations Parris (1970) studied glucose isomerization into fructose using trimethylamine solution. The highest fructose yield of 35 % was achieved when 10 g of glucose diluted in 200 ml of water and 4 ml of trimethylamine at a temperature of 35 °C after 24 hours. In this case no other products were observed besides glucose and fructose. (Parris, 1970) A decade later Hicks (1981) studied the glucose conversion to fructose with the combination of amine and boric acid. The final solution of 63 % of fructose and 37 % of glucose was accomplished with starting solution containing 2,5 mg of glucose and 0,9 mg of boric acid mixed in 100 ml of water which pH was adjusted to about 11 with trimethylamine. Reaction time was 5 hours at 70 °C and also in this case nothing but glucose and fructose products was observed. (Hicks,1981)

More recently Liu et al. (2014) and Carraher et al. (2015) studied glucose isomerization to fructose with amines as a catalyst resulting fructose selectivity between 50−70 % and yields varying between 30−35 %. Liu et al. (2014) investigated organic amines such as morpholine, piperazine, ethylenediamine, triethylamine, piperidine, and pyrrolidine, with pKa between 8,4 and 11,3 which resulted glucose conversions of 43−62 % and fructose yields of 17−31 %

in water solution containing 10 wt-% of glucose after half an hour at a temperature of 100 °C. For example, 31 % fructose yield and 54 % selectivity were reached with 10 mol-% of trimethylamine relative to glucose. (Liu et al., 2014) Further study by Carraher et al. (2015) identified that reaction conditions such as pH had an important role in amine-catalysed conversion of glucose into fructose. Experiments were performed at a temperature of 100 °C in water solution containing 0,52 mol/l of glucose and 0,03−108,68 mol-% of triethylamine (TEA) relative to glucose. Figure 7 shows effect of different initial pH values on fructose formation using trimethylamine as the catalyst with initial pH varying from 9,5 to 11,5. Fructose yields increased from 6 % to 30 % between initial pH value 9,5 and 10,7, and stayed at the same level between pH 10,7-11,3, and then decreased to 23 % at initial pH 11,5. It can be concluded that higher pH values promote fructose formation. (Carraher et al., 2015)

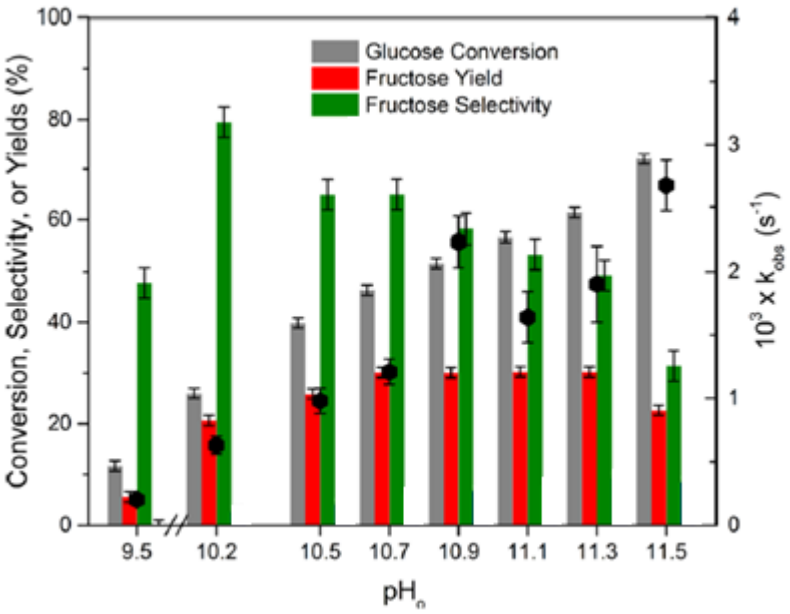

Figure 7 Effect of initial pH on glucose conversion, fructose selectivity and fructose yield when trimethylamine is used to catalyse the glucose isomerization reaction at 100 °C and reaction time was 15 minutes (Adapted from Carraher et al., 2015).

In addition, Carraher et al. (2015) investigated the effect of glucose concentrations performing isomerization reactions with glucose concentrations varying from 0,29–2,9 mol/l. Reactions performed at a temperature of 100 °C with 12 mol-% of TEA relative to glucose which lead to pH of 11. Glucose concentrations between 0,29–0,75 mol/l had little or no effect on gained fructose yield which varied between 23–28 %. However, fructose yield dropped down to 21 % when glucose concentration was increased as high as to 2,9 mol/l. The maximum fructose yield in the presence of trimethylamine in this study of 32 %

was reached at a temperature of 100 °C and pH of 11,3 after 7 minutes solutions containing of 0.52 M glucose. (Carraher et al., 2015)

In addition, Yang et al. (2016) investigated several naturally occurring non-toxic basic amino acids such as arginine, lysine, and histidine as a catalyst to convert glucose into fructose in water solution. These amino acids are nontoxic and therefore they could be environmental friendly and renewable catalysts for glucose isomerization into fructose. Isomerization experiments contained 10 wt-% of glucose diluted in water with amino acids and performed in 6 ml reactors at different temperature ranges varying between 80−130 °C. The experiments allowed to carry on 2−20 minutes. Arginine was observed to be more effective isomerization catalyst compared lysine and histidine. Arginine achieved fructose yield of 31 % at highest with selectivity of 76 % at a temperature of 120 °C, after 15 minutes when at the beginning of the experiment the aqueous glucose solution contained 10 mol-% arginine relative to glucose. (Yang et al., 2016)

Yang et al. (2016) presented that the reaction mechanism of glucose isomerization into fructose catalyzed by arginine takes place via the proton transfer mechanism which is similar as in the case of earlier mentioned strong bases e.g. sodium hydroxide, presented in Figure 3. Both arginine and lysine used in those experiments have amine groups and also contain carboxyl groups. Therefore, it is proposed that arginine and lysine have a similar reaction mechanism to isomerize glucose into fructose as other reported amines. Also, the researchers suggested that the carboxylic acid groups of amino acids were probably the reason for the improved fructose selectivity. This is due to the pH buffering effect of carboxylic acid groups. (Yang et al., 2016)

Solid base catalysts

Besides soluble alkalis, variety of different types of solid base catalysts have been investigated for glucose isomerization into fructose. For example, Delidovich and Palkovits (2014) have studied hydrotalcites with different interlayer anions. Also, zeolites modified with various basic metals such as Li, Na, K, Cs, Ca, and Ba have been experimented (Moreau et al., 2000). In addition, anion-exchanged resins, immobilized amines, metallosilicates and titanates have been studied as a solid catalyst for glucose isomerization into fructose (Lv et al., 2015; Deshpande et al., 2017; Lima et al., 2008; Ohyama et al., 2017).

Anion-exchange resins

Anion-exchange resins have been recognized decades ago as a base-catalyst for glucose isomerization into fructose. Early investigations by Langlois and Larson (1956) reported a maximum yield of fructose to approximately 32 % with hydroxide resin and water as a solvent. In addition, Barker et al. (1975) studied glucose conversion into fructose in sodium hydroxide solution on poly-(4-vinylphenyl boric acid) resin at a temperature of 37 °C and pH adjusted approximately to 12. Outcome of this experiments was a 57 % maximum yield of fructose. (Barker et al., 1975)

Later Rendelman and Hodge (1979) studied glucose conversion into fructose catalyzed with aluminate resin and hydroxide resin in water. The resins were prepared with mixing sodium aluminate ($NaAIO_2$) or sodium hydroxide solution with a strongly basic anion-exchange resin which had a formate group its surface. The highest yields of fructose were accomplished with aluminate resin which had a low hydroxide content (OH^- to sugar ratio lower than one) at pH of about 12 and at temperatures of 2, 13, 23, and 35 °C which gave fructose yields of 72, 68, 62, and 44 %, respectively. It was observed that aluminate resin which had a low hydroxide content resulted better fructose yields than aluminate resin with a normal hydroxide content and also lower temperatures gave better fructose yields. Effect of resin type and reaction time to glucose conversion at 25 °C can be seen from the Figure 8. The highest yield of fructose 28 % was reached in a scheme where hydroxide ions alone were attached to anion-exchanged resin without aluminate ions. (Rendelman and Hodge, 1979)

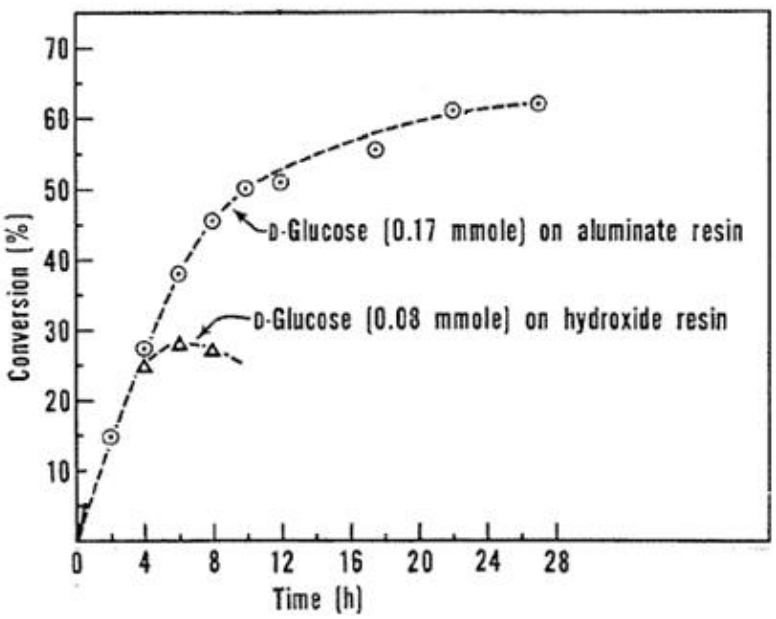

Figure 8 Effect of time and different resin loadings on glucose conversion at 25 °C (Adapted from Rendelman and Hodge, 1979).

In addition, Rendelman and Hodge (1979) carried out kinetic studies of glucose isomerization on aluminate resin which showed that only hydroxide ion is responsible for glucose conversion on aluminate resin. Also, it was observed that the function of the aluminate ion is only to form a complex with fructose. (Rendelman and Hodge, 1979) This is the same phenomenon where aluminate ion forms complex as discussed earlier.

More recent experiments Lv et al. (2015) used strongly basic anion exchange resins namely 201x7, D261, D290 and D296R. These resins have the same functional group of $-N^{+}(CH_3)_3$ and similar physical and chemical properties but only pore size of each resin is different. (Lv et al., 2015; Tianjin Nankai Hecheng Science & Technology Co., Ltd, 2017) Experiments were performed at temperatures of 30, 40, 50 and 60 °C and pH was adjusted to 6, 8, 10 and 12. Effect of glucose to resin ratio was also studied at ratios of 1:1, 1:2, 1:5 and 1:10 g/ml. Each experiment contained 10 ml of resin and 50 ml of aqueous glucose solution and was carried out for 12 h. (Lv et al., 2015)

On one hand, the highest glucose conversion ratio of 73 % was reached at pH 12, at a temperature of 60 °C and glucose to resin ratio of 1:10 but fructose selectivity in this case was only 26 %. On the other hand, highest fructose selectivity of 64 % was result of pH 10, a temperature of 40 °C and glucose to resin ratio of 1:1 but glucose conversion ratio was only about 29 % in this case. Figure 9 presents the effect of reaction temperature and pH

value on glucose isomerization reaction. Overall, it seems that increasing temperature boosts the isomerization reaction towards fructose, but after reaching 50 °C reaction is slightly slowed down towards fructose. Also increasing pH value shows promoting impact on conversion ratio of glucose. (Lv et al., 2015)

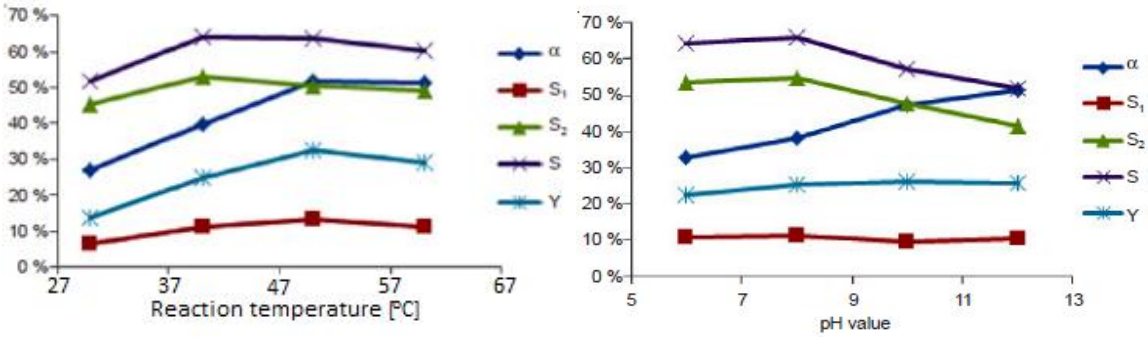

Figure 9 Effect of varying reaction temperature (left) and pH (right) on glucose isomerization into fructose, where α is conversion ratio of glucose, S_1 is mannose selectivity, S_2 fructose selectivity, S total selectivity and Y total yield of mannose and fructose formed (Adapted from Lv et al., 2014).

The glucose isomerization process to fructose utilizing anion exchange resins as catalyst has several advantages such as that there is no need for further operation to separate the end product from catalyst. In addition, the ion-exchange method is more economically suitable compared to traditional enzyme methods. On the other hand, ion-exchange resin as catalyst might also have some disadvantages as hydrothermal instability and difficulties in regeneration. (Zhao et al., 2014)

Amines

Besides soluble catalysts, amines have also been investigated as solid catalysts. When amines are used as a solid catalyst, they are immobilized to onto surface of some solid material such as resin. Yang et al. (2015) carried out experiments with different amines such as IMD (imidazole), API (1-(3-aminopropyl) imidazole), TMG (tetramethylguanidine), TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene), TREN (tis(2-aminoethyl)amine) and PEI (polyethylenimine). The solid catalysts were prepared by chemically immobilizing the amines onto the surfaces of chloromethylated polystyrene resins. For example, 100 mg of glucose and 3 mol-% of TMG relative to glucose in a 1 ml of water achieved fructose yield of 33 % and 73 % selectivity at a temperature of 80 °C after 30 minutes. (Yang et al., 2015)

In the most recent study by Deshpande et al. (2017) a series of mesoporous silica with tertiary amine loadings was investigated as a catalytic material for the glucose conversion into fructose. The catalyst preparation included a synthesis of a tertiary amine group to a surface of a porous SBA-15. The catalyst materials were named after organosilane linker length (C1 vs. C3) and the actual loading. Schematic presentation of organosilane linker length of propyl linker (C3) versus methyl linker (C1) is shown in Figure 10. For example, a silica with methyl linker and with an actual loading of 0,52 mmol/g, the catalyst was labelled to SBA-C3-0.52. (Deshpande et al., 2017)

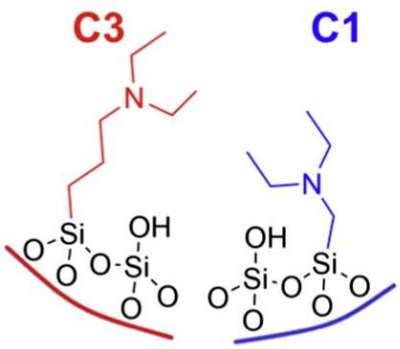

Figure 10 Organosilane linker length of C3 (left) and C1 (right) on the surface of mesoporous silica (Deshpande et al., 2017).

According to Deshpande et al. (2017) benefits of this kind of catalysts are high selectivity of tertiary amines, easy preparation and high porosity. Experiments were carried out using 10 mol-% tertiaryamine loading and a 10 wt-% glucose solution at 100 °C. SBA-C1-0.95 gave the highest fructose yield of 23 % combined with a high fructose selectivity of 75 %. It was observed that surface silanols played a significant role to the catalyst performance. It was found during the experiments that isomerization with similar actual loadings, for example SBA-C1-0.52 and SBA-C3-0.52, the catalyst with longer linker length was less active and selective than the catalyst with shorter linker length. The basicity of amine can be increased by the close proximity of the amine to silicon atom. Figure 11 shows the effect of linker length (C1 and C3) to glucose conversion and fructose selectivity as a function of time. Shorter linker length of the active site but also larger surface concentration of the active site seemed to promote the catalyst activity. (Deshpande et al., 2017)

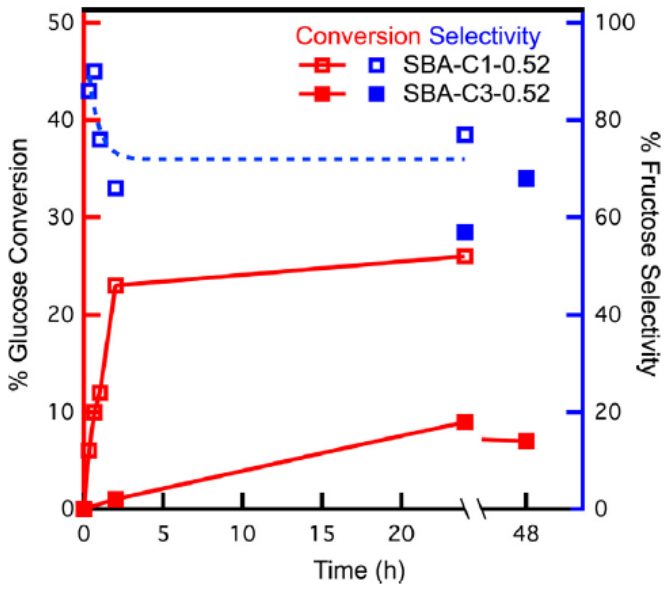

Figure 11 Effect of linker length (C1 and C3) to glucose conversion and fructose selectivity with the same actual loading of 0,52 mmol/g (Deshpande et al., 2017).

Yang et al. (2016) have investigated polystyrene-supported organic amines with varying structural properties such as ethoxylated, linear and branched polyethylenimines (PEIs) as a catalyst for glucose isomerization. Compared with other amine catalysts, PEIs are more environmentally friendly catalyst due its relatively lower toxicity and odorless. They are also more efficient because it is easier to separate and recycle in the process than other amine catalysts. The PEIs utilized in these experiments were polyamines, therefore it is assumed that the PEIs have a similar isomerization reaction mechanism to amines such as triethylamine. (Yang et al., 2016)

Investigating PEIs as a catalyst to glucose isomerization, the effect of different glucose concentrations, PEI dosage, retention time, and temperature was investigated. 6 ml of aqueous glucose solution of 10−50 wt-% was carried out at different temperatures varying between 80−120 °C. The reaction was allowed to carry on for 1 to 25 minutes. Higher glucose conversion and fructose yields were observed when increasing the PEI dosage, reaction time, and temperature. However, higher glucose concentration was found give lower fructose yields. The branched PEI showed the highest catalytic activity after 10 minutes at 120 °C resulting 33 % fructose yield and 77 % selectivity a catalyst dosage was 0,05 mol % relative to glucose. (Yang et al., 2016)

As mentioned earlier, amines can generate hydroxide ions in water, which increase alkalinity of a glucose solution and then acts as a base catalyst. However, the hydroxide ion generation from amine in water can be affected by the addition of a neutral salt. (Yang and Runge, 2016) For example, effect of neutral salts, such as NaCl, KCl, LiCl, NaBr, LiBr, NaI and KI, on the glucose isomerization reactions in the presence of PEI was experimented by Yang and Runge (2016). Even as small addition as 1 wt-% of neutral salts, glucose isomerization in presence of PEI resulted about fructose yield of 41 % and fructose selectivity between 72−78 % at 110 °C after 12 minutes. However, increasing the concentration of neutral salt from 1 wt % to 20 wt % did not result better fructose yields or selectivity. (Yang and Runge, 2016)

Improved yield of fructose is due to that the specific bindings between anions of salts, such as Cl^-, Br^-, and I^-, with the positively charged sites of the PEI chains can shift the reversible hydrogen generation reaction equilibriums more in the side of hydroxide ions. In other words, when the protonated amine and the anion of salt forms a complex with each other through electrostatic interaction, the complex is more stable which shifts the reaction equilibrium towards hydrogen production and increases alkalinity of the amine solution. Figure 12 shows a schematic presentation of the reaction between PEI, water and salt which creates hydroxide ions. (Yang and Runge, 2016)

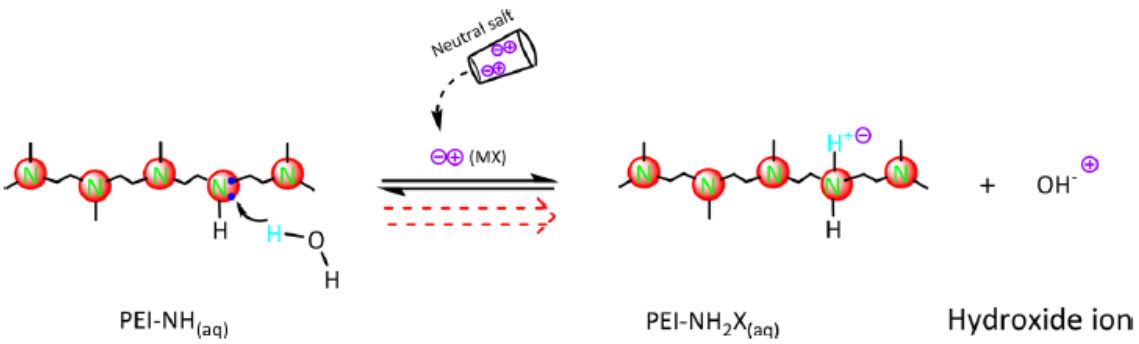

Figure 12 Addition of neutral salt can shift the equilibrium of protonation reaction between PEI and water towards hydroxide ion production. M = Li^+, Na^+ or K^+ and X = Cl^-, Br^- or I^-. (Yang and Runge, 2016)

Zeolites

Cation-exchanged zeolites have been shown catalytic activity in the glucose isomerization reaction to fructose. (Moreau et al., 2000) Zeolites are crystalline aluminosilicates with an ordered crystalline structure consisting of $[AlO_4]^{5-}$ and $[SiO_4]^{4-}$. In the case of cation exchanged zeolites, the cations are placed in the way that the negative charge on the surfaces

of zeolites is balanced. Simplified structure of zeolite in a Na^+ exchanged for is shown in Figure 13. (Jha and Singh, 2016)

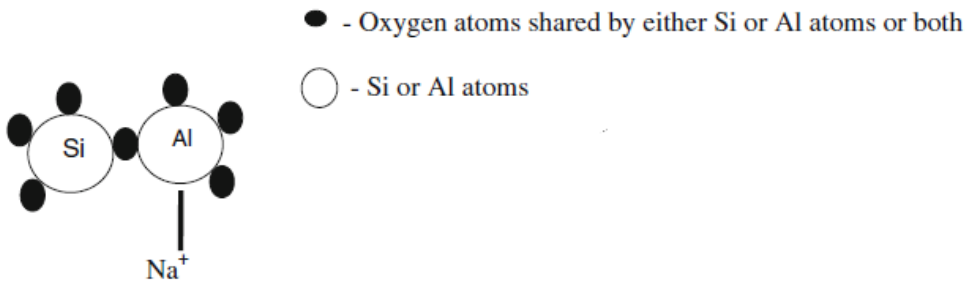

Figure 13 Simplified structure of a Na^+ exchanged zeolite (Jha and Singh, 2016).

Experiments by Moreau et al. (2000) glucose conversion into fructose was performed in the presence of various cation-exchanged A, X and Y zeolites. Used exchanged cations were Li, Na, K, Cs, Ca and Ba. Cation exchanged zeolites were prepared from commercially available zeolites which were in a sodium-form, namely NaA, NaX and NaY. These zeolites were exchanged with the one of previously mentioned cation. In all experiments, an autoclave was filled with 5 g of glucose, 1 g of catalyst and 50 ml of water and carried out at a temperature of 95 °C. Divalent cations such as Ca- and Ba-exchanged zeolites were found less selective, compared with monovalent cations such as Li, Na, K and Cs. The highest fructose selectivity of 52 % with divalent cation exchanged zeolite was achieved with BaX zeolite catalyst but in this case glucose conversion was only 12 %. While highest fructose selectivity of 86 % was reported with monovalent cation, namely NaX. However, then glucose conversion was only 20 %. Catalytic activity considering exchanged cation was reported as follows: Ca^{2+} < Ba^{2+} <Li^+ <Na^+ <K^+ <Cs^+. Lower activity of divalent exchanged zeolites can be explained by the fact that the presence of divalent cations has a lower basic character than monovalent exchanged zeolites. (Moreau et al., 2000)

Notably, a significant cation leaching was observed when zeolites loaded with monovalent cation (Li, Na, K and Cs) was used. Approximately even 12–30 % of cations of the zeolite leached to the water. However, no cation leaching was observed in the case of divalent cations (Ca and Ba). (Moreau et al., 2000) It is speculated that monovalent cation of the zeolite is replaced by hydronium ion as compensating cation when water is used as a solvent. Cation leaching reduces the basicity of the zeolite. (Graca et al., 2017)

Hydrotalcines

Magnesium–aluminum hydrotalcites have been also investigated as catalysts for glucose isomerization into fructose. Hydrotalcites are layered double hydroxides and have a general formula of $[Mg_{1-x}Al_x(OH)_2]^x+(A_{x/n}{}^{n-})\cdot mH_2O]$ where A^{n-} is an interlayer anion, x presents the fraction of aluminium which vary from 0,17 to 0,33, and m stands for water molecules of crystallization. (Cavani et al., 1991) Figure 14 shows a schematic presentation of hydrotalcite structure (Palmer et al., 2009). The interlayer anions have known to act as active species for the glucose conversion reaction in the hydrotalcites. (Lee et al., 2014). Particulary, hydrotalcites which have HO^- or CO_3^{2-} as an interlayer anion have been studied for glucose conversion into fructose. Generally, hydrotalcites consisting of HO^- as an interlayer anion have shown more activity compared to hydrotalcites consisting of CO_3^{2-} as an interlayer anion because the basicity of HO^- is tend to be higher than of CO_3^{2-}. (Lecome et al., 2002; Nishimura et al., 2013)

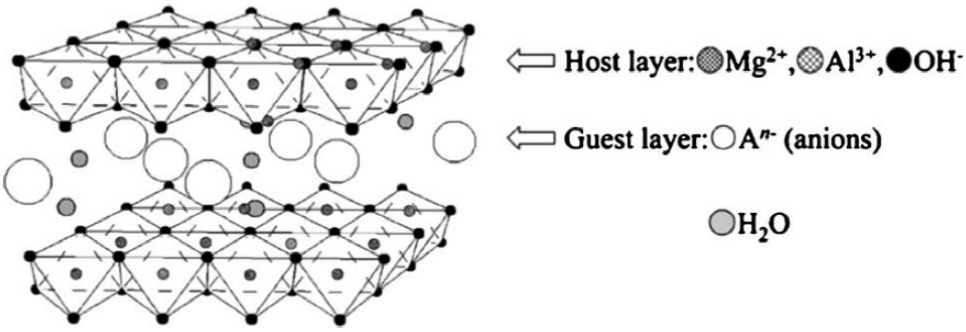

Figure 14 Schematic presentation of a general hydrotalcite structure (Palmer et al., 2009).

Delidovich and Palkovits (2014) have studied one hydrotalcite which surface was modified to hydrophobic and two hydrophilic hydrotalcites with CO_3^{2-} or OH^- anion as an interlayer specie to catalyse glucose isomerization into fructose. Experiment carried out 30 ml containing 1 wt-% of aqueous glucose solution and 1 g of catalyst at a temperature of 90 °C and resulted glucose conversion of 41 %, fructose selectivity of 79 %, and yield of 32 % with hydrotalcite in carbonate form after 24 h. While at the same reaction conditions hydrotalcites with a hydroxide anion as an interlayer specie resulted glucose conversion of 71 %, fructose selectivity of 49 % and yield of 33 %. With these reaction conditions both the carbonate and the hydroxide anion exhibit comparable activity. On the one hand hydroxide anion as an interlayer of hydrotalcite gives a better conversion rates than carbonate form hydrotalcite but on the other hand fructose selectivity is slightly improved

with hydrotalcite in a carbonate form compared with hydroxide form. (Delidovich and Palkovits, 2014)

In addition, Yu et al. (2012) and later Lee et al. (2014) have studied glucose isomerization into fructose with hydrotalcite with hydroxide anion as an interlayer specie as a catalyst and dimethylformamide (DMF) as a solvent. Experiments by Yu et al. (2012) was performed at a temperature of 80 °C and 0,1 g of catalyst and 0,3 g of glucose was added to 10 ml of DMF. After three hours 69 % fructose selectivity and 35 % yield were obtained. Furthermore, the effect of reaction temperature on glucose isomerization with hydrotalcite was studied by performing the reaction at temperatures varying between 70−110 °C. Figure 15 present the change after five hours in conversion ratio of glucose, fructose selectivity and yield as a function of reaction temperature. It can be seen that as the temperature increases, conversion of glucose was increased while selectivity for fructose was decreased. In addition, the highest fructose yield was reached at a temperature of 80 °C. (Yu et al., 2012)

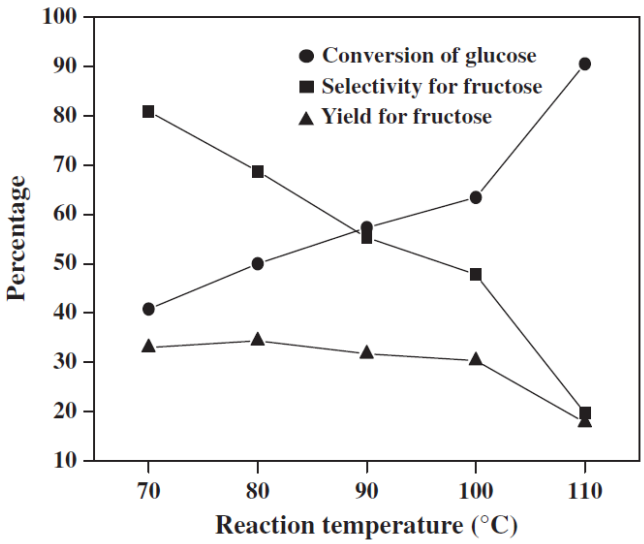

Figure 15 Glucose conversion, fructose selectivity and yield changes as a function of reaction temperature when hydrotalcite with hydroxide anion as an interlayer specie was used as a catalyst and DMF as a solvent. (Yu et al., 2012)

Similar experiments with Yu et al (2012) was carried out later by Lee et al. (2014). Additionally, Lee et al. (2014) utilized sonication method for pre-treating hydrotalcites during rehydration. The aim of sonication is to increase the catalytic activity of rehydrated hydrotalcite in the glucose isomerization into fructose. Sonication causes vertical breaking and exfoliation of the hydrotalcite layers. This result to an increased concentration of basic centres and in that way increases catalytic activity of hydrotalcite. A variety of rehydrated

hydrotalcites with varying sonication time from 5 to 60 minutes was investigated. Experiments were performed in a glass reactor containing 0,3 g of glucose and 0,1 g of catalyst in 10 ml of DMF at a temperature of 100 °C. After three hours the highest yield of fructose 37 % and selectivity 88 % was obtained using hydrotalcite with sonication time of 10 minutes. (Lee et al., 2014)

Metallosilicate

Lima et al. (2008) have tested series of metallosilicate materials such as titanosilicates (ETS-10, ETS-4 and AM-4), a sodium yttrium silicate (AV-1), an alkali calcium silicate (AV-2), and a calcium silicate (TOB) as solid base catalysts for glucose conversion into fructose. Experiments were conducted in a glass reactor at 100 °C containing 50 mg of glucose, 20 mg of catalyst and 1 ml of water. All the tested metallosilicate materials gave fructose yields between 20–40 % after two hours. For example, highest yield of fructose was obtained with titanosilicate ETS-4 (unit cell formula $Na_9Si_{12}Ti_5O_{38}(OH)$) which obtained fructose yield of 39 % and selectivity of 84 %. For comparison, the calcium silicate TOB (unit cell formula $Ca_5Si_6O_{17}$) obtained fructose yield of 35 % and selectivity of 69 %. Occasionally, small amounts of mannose was observed in less than 2 % yield. (Lima et al., 2008)

In addition, Lima et al. (2008) carried out the Knoevenagel condensation characterization tests where the aim was to investigate the base properties of the alkaline metallosilicates. It was observed that ETS-4 had the highest basicity, while TOB had the second lowest basicity. The following order of basicity was observed: ETS-4> AM-4 and AV-1> AV-2 and TOB> Na-ETS-10. It was noticed that this trend correlates quite well with the obtained fructose yields from isomerization reaction. It can be concluded that the base properties play a key role in glucose conversion into fructose. (Lima et al., 2008)

Titanates

Ohyama et al. (2017) have investigated very recently series of titanates as a catalyst for the glucose isomerization. Studied titanates were $SrTiO_3$, $BaTiO_3$, $CaTiO_3$, $Na_2Ti_6O_{13}$, $K_2Ti_6O_{13}$, and $Sr_3Ti_2O_7$. Experiments were carried out at a temperature of 110 °C and reaction time of one hour including 2 ml of solution which glucose content was 10 wt-% and 0,2 g of catalyst. Isomerization with $SrTiO_3$, $CaTiO_3$, and $Na_2Ti_6O_{13}$ catalysts obtained the

highest fructose yield of 32 % and fructose selectivity was in that case around 68–78 %. (Ohyama et al., 2017)

Furthermore, the base quantity and strength was evaluated to investigate the effect of basicity of titanates on glucose conversion into fructose. The base amounts of the titanates were evaluated by using a titration method. It was observed that the glucose conversion as well as the fructose yield was increasing with the higher base amounts. In addition, determination of base strength was carried out with an acid–base titration and Fourier-transform infrared spectroscopy (FTIR). The following order of the base strength of titanates was arisen $BaTiO_3$ <$SrTiO_3$ <$K_2Ti_6O_{13}$ <$Na_2Ti_6O_{13}$ <$CaTiO_3$ <$Sr_3Ti_2O_7$. It was noticed that the stronger the base was the higher the catalytic activity, correspondingly. The strong basicity of $Sr_3Ti_2O_7$ can be considered as a possible reason for the high catalytic activity of $Sr_3Ti_2O_7$. Conversely, the weakest base $BaTiO_3$ had lower catalytic activity in glucose isomerization reaction. Therefore, it can be concluded that both the base strength and the base amount of solid base catalysts affects to glucose conversion into fructose. (Ohyama et al., 2017)

2.2.2 By-product formation in alkaline conditions

The use of strong bases, such as sodium hydroxide, calcium hydroxide or potassium hydroxide, combined with alkaline conditions and high operation temperature, glucose isomerization suffers from a low yield of fructose due to the formation of numerous acidic by-products and colored substances of unknown structure. (Zhao et al., 2014) Besides the isomerization reaction, degradation of monosaccharides into acidic products is also catalyzed by bases. Strong bases are known to catalyze the degradation of monosaccharides into carboxylic acid compounds, e.g. saccharinic acids, lactic acid or formic acid. (Bruijn et al., 1987) Degradation of monosaccharides into acidic compounds leads to neutralization of the basic catalyst. In addition, dehydration and condensation of acidic compounds leads to a strong darkening of the sugar solution which is problematic especially in the food industry. (Hanover and White, 1993; Delidovich and Palkovits, 2016) In addition to low yields of fructose, separation of the acidic by-products from a sugar solution decreases the process efficiency and cost-efficiency. (Zhao et al., 2014)

Generally, acidic by-product formed from monosaccharides under alkaline conditions can be divided into those acidic compounds which have the same carbon skeleton of the parent

sugar and those which have a shorter carbon chains. For example, saccharinic acids, which are common product formed from monosaccharides under alkaline conditions, are acidic compounds which have the same carbon chain of the original sugar. This kind of acidic compounds are formed via benzilic acid type rearrangement of the corresponding deoxy sugars. Acidic compounds which have a shorter carbon chain than the original sugar is formed either when sugar intermediates split directly or when the carbonyl fragments undergoes subsequent reactions. (Novotný et al., 2008)

Yang and Montgomery (1995) investigated glucose degradation in alkaline conditions. In the experiments glucose was diluted in an aqueous calcium hydroxide ($Ca(OH)_2$) where concentration of both glucose as well as calcium hydroxide was 0,1 mol/l. The experiment was carried out for 30 minutes at 100 °C which led to a mixture of over 50 compounds and glucose was completely degraded. The pH of the solution decreased from 13 to 8,3 during the experiment, which could be caused by formation of acidic compounds. Detected reaction products consisted mainly of various saccharinic ($<C_6$) acids and the principal acidic product was lactic acid. Some compounds were detected in smaller amounts, such as propanoic acid, 2-hydroxybutanoic and the 2- and 3-deoxy-hexonic and -pentonic acids. (Yang and Montgomery, 1996)

Additionally, effect of the concentration of reactants on glucose degradation into acidic products was investigated. An equimolar ratio of glucose and calcium hydroxide was kept the same but the concentration of glucose was varying between 1,8−100 w-%. Varying concentration of reactants resulted to the same saccharinic acids but in different proportions. The increase of glucose concentration promoted more the formation of C_6 acids (such as 2-C-methylpentonic, hexametasaccharinic, and isosaccharinic acids) and decreased the formation of $<C_6$ acids (such as glycolic, lactic, glyceric, 2-C-methylglyceric, deoxytetronic, and deoxypentonic acids). (Yang and Montgomery, 1996)

It can be speculated that using strong bases as a catalyst for the isomerization of glucose into fructose that both of these monosaccharides are degraded to acidic compound since both are unstable under hot alkaline conditions. Fructose might be even more degraded during isomerization due it is even more unstable under alkaline conditions than glucose (Sigma-Aldrich, 1997; DOW, 2017).

2.2.3 Acid catalysts

Glucose isomerization into fructose with acids as a catalyst was discovered way later than basic catalysts. This is probably due to the fact that classic Lewis acids, for example aluminum chloride ($AlCl_3$) is usually not active in the presence of water. (Roman-Leshkov and Davis, 2011) The negative effect of water can be reduced by combining the Lewis acid site (for example Sn or Ti atoms) to a material which is relatively hydrophobic such as zeolite or porous silica (Gounder and Davis, 2013).

Solid acid catalysts

The reaction mechanism of glucose isomerization into fructose is somewhat different in the case of acid catalyst and base catalyst. As mentioned in section 2.2.1, the base catalyzed glucose isomerization in water follows the proton transfer mechanism creating an enediol intermediate but in the case of acid catalyst it follows so called intramolecular hydride shift mechanism. Figure 16 shows a schematic presentation of glucose isomerization reaction mechanism into fructose when acid is used as a catalyst. In the first step the acid sites i.e., metal centers (in this case Sn) coordinates with oxygen of the carbonyl group of the glucose molecule through its lone electron pair which causes the polarization of the C=O bond. At the same metal center coordinates with O2 atom which lead to form a five-membered cyclic complex. The complex promotes the intramolecular hydride shift between the C2 and C1 positions, eventually leading to the formation of fructose. (Roman-Leshkov and Davis, 2011; Corma et al., 2001)

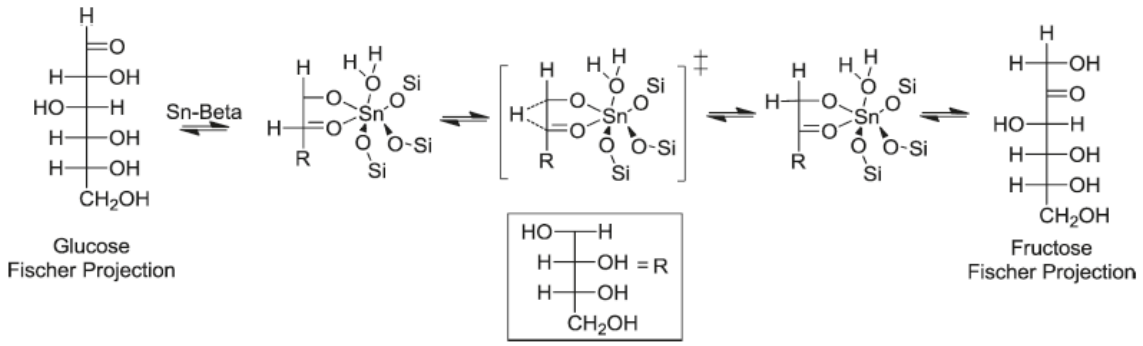

Figure 16 Reaction mechanism of glucose conversion into fructose using solid Lewis acid as a catalyst (Roman-Leshkov et al., 2010).

Moliner et al. (2010) studied a large pore zeolite that contained either tin (Sn) or titanium (Ti) metal centers as a catalyst for glucose conversion into fructose in water. Reactions were

carried out at a temperature of 110 °C with a 10 wt-% of glucose solution and amount of catalyst which reached a 1:50 metal to glucose molar ratio. After half an hour product yields of approximately 45 % glucose, 32 % fructose, and 9 % mannose was observed with Sn-Beta as a catalyst. At the same reaction conditions but with longer reaction time of 90 minutes, the Ti-Beta catalyst achieved much lower fructose yield of 14 %, and higher glucose yield of 74 %. (Moliner et al., 2010)

Generally, experiments of glucose isomerization into fructose utilizing acid catalysts, such as Sn-, Ti-, and Zr-containing silicate materials, and water as a solvent has resulted lower yields of fructose than 40 %. Interestingly, Saravanamurugan et al. (2013) have investigated glucose isomerization into fructose utilizing aluminosilicate as a catalyst and besides water, also alcohol as a solvent. Experiments were carried out using zeolite catalysts which contained only silicon and aluminum. The experiment consisted two reaction steps. At first, glucose was isomerized into fructose in methanol where a part of fructose reacted into methyl fructoside. Equilibrium shifts more towards glucose due part of fructose is in the form of methyl fructoside. In a last reaction step, water was added which hydrolyzed the methyl fructoside creating more fructose. Figure 17 shows a simplified schematic presentation of two-step glucose isomerization reaction mechanism via methyl fructoside into fructose. (Saravanamurugan et al., 2013)

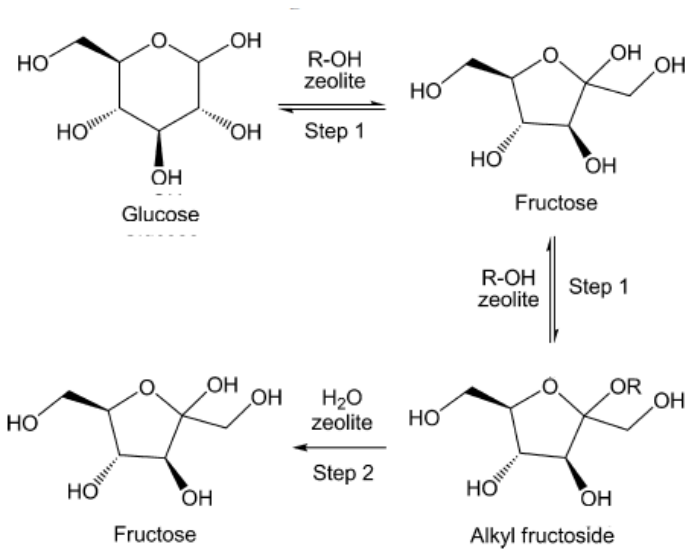

Figure 17 Two steps involving reaction pathway to form fructose from glucose in alcohol and in water (Saravanamurugan et al., 2013).

This two-step process was carried out under 120 °C and both reaction steps was allowed to carry out for one hour. In the first step, reaction mixture contained 75 mg of catalyst, 125

mg of glucose and 4 g of methanol. In the reaction step 2 reaction was allowed to proceed another hour after addition of 4 g of water. H-USY zeolite which Si/Al ratio was 6 showed the highest catalytic activity. These reaction conditions lead to a fructose yield of 55 % and an 76 % fructose selectivity at highest. (Saravanamurugan et al., 2013)

Later in 2016 Saravanamurugan et al. investigated more deeply the function of the catalyst active sites in this two-step isomerization reaction. It turned out that the zeolites function as bifunctional catalysts and works as Lewis-acid-catalyst for isomerization of glucose into fructose and also as Brønsted-acid-catalyst for trapping of fructose into fructosides. The zeolites acting as bifunctional catalyst result kinetic product control and higher equilibrium yields of fructose after hydrolysis. (Saravanamurugan et al., 2016)

Soluble acid catalysts

Also, soluble Lewis acids has been investugated as catalysts for glucose isomerization into fructose. For example, Tang et al. (2015) have studied molecular salts, such as $CrCl_3$, $AlCl_3$ and $SnCl_4$, for glucose conversion into fructose. The catalytic activity of these molecular salts was proven to decrease in an order of $CrCl_3$>$AlCl_3$>$SnCl_4$. Choudhary et al. (2013) and Mushrif et al. (2012) have studied the isomerization reaction mechanism with $CrCl_3$ as a catalyst and water as a solvent and have proven that a partially hydrolyzed $[Cr(H_2O)_5(OH)]^{2+}$ cation act as an active species and is then responsible for the conversion of glucose into fructose. In addition, Tang et al. have proven that $[Al(OH)_2aq]^+$ cations are the active centers in glucose-fructose conversion in the presence of $AlCl_3$ in water. (Tang et al., 2015)

Choudhary et al. have recognized a similar kind of reaction mechanism for the glucose isomerization into fructose over solid (such as Sn-beta) and soluble (such as $AlCl_3$ and $CrCl_3$) Lewis acids. (Choudhary et al., 2013) Analogously to catalysis using Sn-beta as a catalyst in solid media, isomerization of glucose over soluble Lewis acids proceeds by so called “hydride shift” reaction mechanism. (Tang et al., 2015; Choudhary et al., 2013; Mushrif et al., 2012) At first, glucose deprotonates with one of the hydroxyl groups attached to the metal center. Then hydroxyl groups attached to C1 and C2 atoms of glucose replace two water molecules which are attached to the metal center. In the last step the glucose molecule receives the proton back from the intermediate water molecule of metal complex by water mediated proton transfer and the final form of fructose molecule is reached. Figure

18 shows a schematic presentation of glucose isomerization into fructose with a soluble acid catalyst in aqueous solution. (Mushrif et al., 2012; Tang et al., 2015; Choudhary et al., 2013)

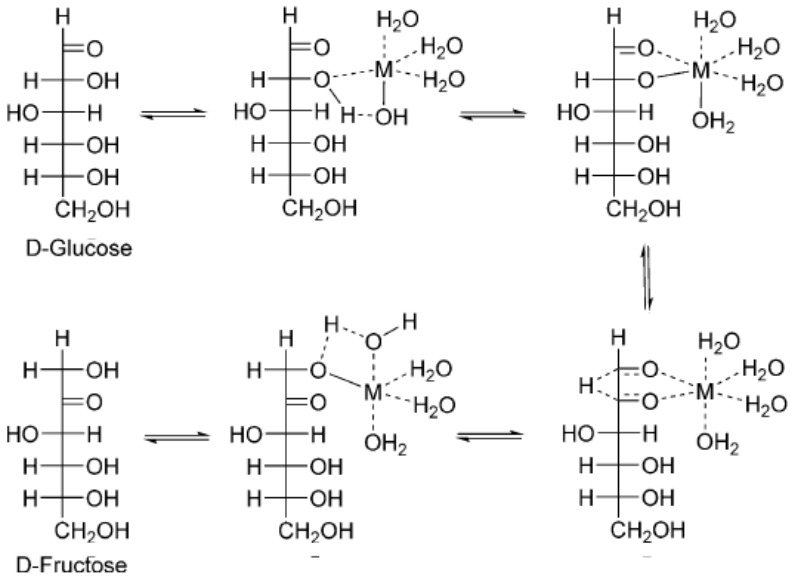

Figure 18 Glucose isomerization into fructose proceeds via “hydride shift” reaction mechanism in the presence of soluble Lewis acids. Here “M” represents metal ion, e.g., Cr^{2+}, Al^{2+}. (Tang et al., 2015; Choudhary et al., 2013; Delidovich and Palkovits, 2016)

Choudhary et al. (2013) investigated $CrCl_3$ to catalyze glucose isomerization in aqueous media. Experiments were performed with 10 wt-% solution of glucose and Cr to glucose molar ratio of 3:100 at a temperature of 140 °C. After half an hour, fructose yield of about 20 % and glucose conversion of approximately 50 % was observed. Figure 19 shows the glucose conversion and fructose yield of a function of time using $CrCl_3$ as a catalyst in glucose-water solution. It can be seen that after around 30 minutes the fructose concentration reaches its maximum, and after that other products such as HMF, levulinic acid, and formic acid are detected. (Choudhary et al., 2013)

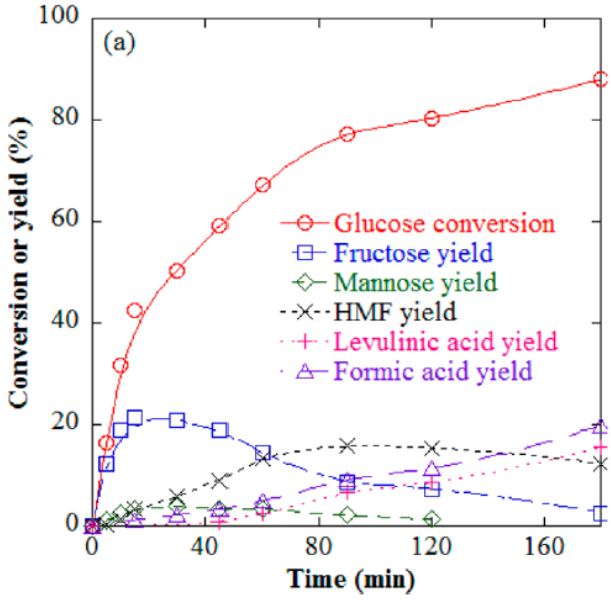

Figure 19 Glucose isomerization into fructose utilizing $CrCl_3$ as a catalyst in water at a temperature of 140 °C where Cr to glucose molar ratio was 3:100 (Choudhary et al., 2013).

Very recently Yoo et al., (2017) investigated glucose isomerization to fructose with five different salt hydrates diluted in water, namely LiCl, LiBr, LiI, $CaBr_2$, and $ZnCl_2$. Experiments was carried out in a 20 ml glass reactor with stirring where reaction mixture contained 50 mg of glucose and 5 ml of the salt hydrate at 120 °C. After 15 minutes 31 % of glucose was converted to fructose by LiBr as a catalyst. It was noticed that the bromides LiBr and $CaBr_2$ which gave fructose yields of 30 % and 22 % respectively and were significantly more efficient than the chlorides LiCl and $ZnCl_2$ which gave fructose yields of 8 % and 0 %, respectively. In addition, considering cations Li^+ seemed to perform better than Ca^{2+} and Zn^{2+}. (Yoo et al., 2017)

Furthermore, Yoo et al. (2017) investigated reaction mechanisms of glucose isomerization into fructose with isotopic labeling experiments. LiBr only was investigated due it showed the best performance catalyzing the conversion of glucose into fructose compared to other salts investigated. Studies proved that isomerization reaction had two possible reaction pathways “A” and “B” which are presented in the Figure 20. In path A, Br^- grabs the hydrogen of C2 of glucose molecule, which leads to a formation of an enediol intermediate. Fructose is finally formed from the enediol intermediate by a proton transfer from the LiBr solution to C1. In path B, a similar intramolecular “hydride shift” transfer occur as mentioned earlier with metal cations, which is in this case Li^+. (Yoo et al., 2017)

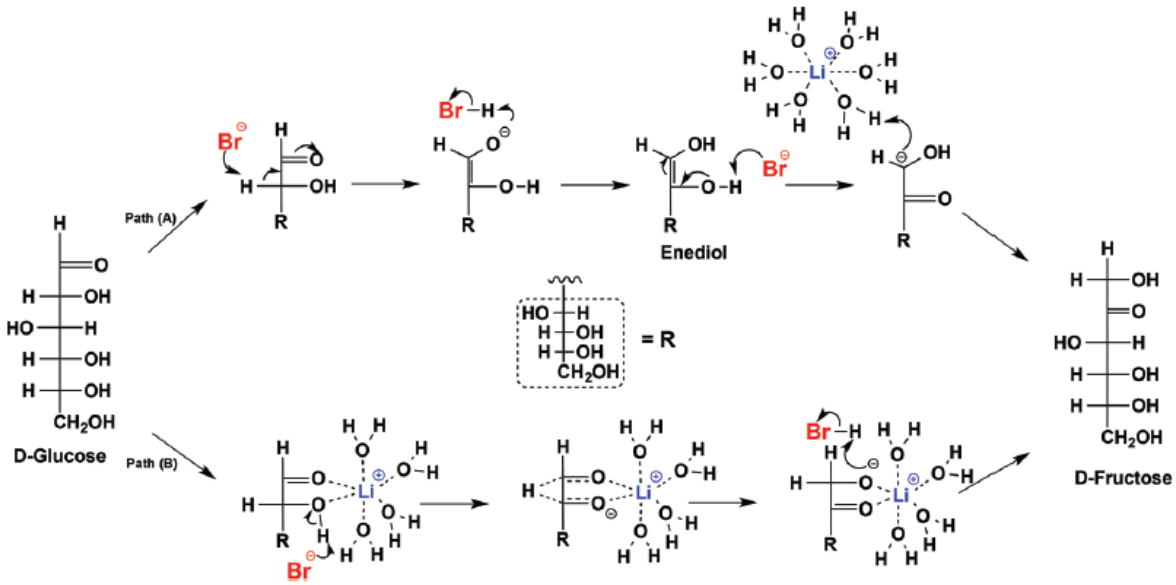

Figure 20 Proposed reaction mechanism to glucose-fructose conversion by LiBr catalyst in water. Path A is presented on top and path B on bottom. (Yoo et al., 2017)

In conclusion, it was confirmed that both the cation (Li^+) and also the anion (Br^-) of the salt hydrate took part in catalyzing the glucose conversion into fructose and that the reaction was able to took both proton transfer (path A) and hybrid shift (path B) reaction pathways. However, the researchers proposed that path A was more dominant than path B for glucose conversion in the salt hydrate solution. Path A was responsible about 85 % of the conversion reaction and path B was only 15 %. (Yoo et al., 2017)

2.3 Enzymatic catalyst

As mentioned earlier, glucose isomerization into fructose is already an industrial scale and commercial process. D-Glucose/xylose isomerase (EC 5.3.1.5) also commonly known as glucose isomerase (GI), is used to catalyze the isomerization reaction of glucose into fructose and it was discovered in the mid-1900s. (Tsumura and Sato, 1965; Khalilpour and Roostaazad, 2008; Yubin et al., 1998.) The enzymatic isomerization of glucose into fructose is used especially in the production of high fructose corn syrup (HFCS). Clinton Corn Processing Co in 1967 was the first company who succeed in enzymatic isomerization of glucose into fructose in industrial scale at the first time. Immobilized GI was introduced at the first time later in 1974. The demand of HFCS increased over time and by 1980 most of starch-processing companies relied on enzymatic isomerization of glucose into fructose utilizing GI. (Bhosale et al., 1996). Nowadays glucose isomerase has the largest market as

an immobilized enzyme for production of HFCS because the enzymatic process is highly efficient and very selective. (Ray and Rosel, 2017; Kent, 2012) Globally, this process uses approximately 1500 tons of immobilized GI and produces over 10 million tons of HFCS (dry substance) per year. (Buchholz and Seibel, 2008; Misset, 2003)

Many organisms are glucose isomerase producers. Micro-organisms such as Streptomyces, Bacillus, Arthobacter and Actinoplanes species can be used for glucose isomerase production. (Saha et al., 2009) Glucose isomerase is generally produced by submerged aerated microbial fermentation. Fermentation typically takes about a couple of days but the reaction conditions may vary from producer to another. (Bhosale et al., 1996; Kent, 2012) The desirable application conditions of immobilized glucose isomerase in bioreactors are usually at pH 7–8 and 60–65 °C to control microbial growth. Immobilized GI is capable to produce a 42 % fructose isosyrup. (Misset, 2003; Kent, 2012) Usually fructose isosyrup is concentrated to 55 % of fructose content utilizing chromatographic separation. (Birch et al., 2012)

In comparison with process using soluble GI, advantages of an immobilized GI process are greater amount of gained product per unit of enzyme due the same enzyme is used continuously. In addition, the isomerization process is possible to carry out continuously and being automated, and there is no need for extra process to separate the catalyst from the product. (Misset, 2003; Kent, 2012) However, the enzymatic glucose isomerization process has also some limitations such as requirement of extremely high purity of glucose and the process conditions have to be carefully kept within a very limited temperature and pH range. If process conditions are not controlled well a result might be lower selectiveness and the enzyme might deactivate through microbial growth or thermal degradation. (Souza et al., 2012; Mushrif et al., 2014)

In the case of enzymatic glucose isomerization into fructose, it is generally believed that glucose isomerase acts as in the acid-catalyzed isomerization where a metal center assists the “hydride shift” reaction pathway (Collyer and Blow, 1990). This is due to glucose isomerase being a metalloenzyme and requires Mg^{2+}, Mn^{2+} or Co^{2+} for its activity (Misset, 2003). The isomerization reaction mechanism in the presence of glucose isomerase is shown in Figure 21. Similarly, with the acid-catalyzed glucose isomerization, enzymatic isomerization includes ring opening and isomerization through two Mg^{2+} mediated hydride

shift from C2 of glucose molecule to C1 to form fructose and lastly the ring closure. (Lavie et al., 1994)

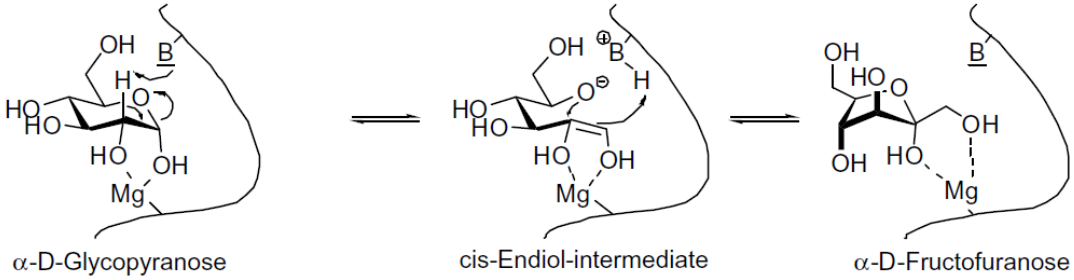

Figure 21 Glucose isomerase used as a catalyst converts glucose into fructose via "hydride shift" mechanisms (Collyer and Blow, 1990; Lavie et al., 1994).

2.4 Summary

Glucose can be isomerized into fructose by chemical or enzymatic catalysts. Chemical catalysts can be further classified to base and acid catalysts which may be either in a solid or a soluble state. Generally, it can be concluded that reaction temperature, time, solution pH and glucose concentration have an effect on glucose isomerization into fructose but also glucose and/or fructose degradation into acidic products. The enzymatic glucose isomerization into fructose is a well-known industrial-scale process which has to be operated within very limited pH and temperature range, commonly at pH 7–8 and 60–65 °C, due to fructose selectivity. However, an ideal process conditions and chemical catalyst have not been discovered for a chemo-catalytic system although glucose conversion into fructose has been intensively studied for hundreds of years. Table V presents an overview of relevant literature in this study of glucose isomerization over solid and soluble chemical catalysts at different reaction conditions.

The main focus in this study is on investigating glucose isomerization and degradation reactions under alkaline conditions. Regardless of the catalyst, more alkaline conditions seem to promote glucose conversion to fructose but also to acidic compounds. For example, critical pH for fructose selectivity was pH 8 in experiments where strongly basic anion exchange resin catalyst was used, in section 2.2.1 carried out by (Lv et al., 2015) and pH 10−11,5 in experiments where triethylamine was used as the catalyst carried out by (Carraher et al., 2015). Overall based on literature findings, it seems to be worth exploring at least pH range between 8−11 when investigating glucose isomerization.

Glucose isomerization experiments introduced in this literature review have been usually carried out at relatively high reaction temperatures, most of them between 80−130 °C. Overall, it seems that increasing temperature promote the isomerization reaction of glucose into fructose. However, catalytic systems which require higher reaction temperatures cost more and are more energy consuming. Downside of higher reaction temperatures is also glucose degradation to acidic compounds. Glucose and especially fructose are unstable under alkaline conditions combined with higher reaction temperatures. Table V presents only glucose isomerization experiments carried out at temperatures which are 100 °C or lower. It can be seen that higher reaction temperature is not always required to reach a good fructose yield. For example, in the experiments carried out by Rendelman and Hodge (1979) and Langlois and Larson (1956) in the section 2.2.1., glucose is successfully isomerized into fructose at reaction temperatures even as low as 25−35 °C. Based on literature data, reaction temperature between 50−60 °C should be suitable for glucose isomerizing into fructose without significantly reducing the selectivity of fructose. Therefore, effect of reaction temperature on glucose isomerization between 20−60 °C would be proper to investigate.

Reaction time of glucose isomerization experiments introduced in this literature review have varied from 10 minutes to several days. Generally, fructose yield increases with longer reaction time but some studies have shown that longer reaction time than 6 hours have only little or no effect to glucose conversion or fructose yield. For example, Deshpande et al. (2017) investigated glucose isomerization with a series of mesoporous silica containing tertiary amine loading, introduced in section 2.2.1. Glucose conversion or fructose selectivity hardly changed after 5 hours. Also, extending reaction time from 24 h to 48 h had no effect. (Deshpande et al., 2017) Overall, it seems that glucose can be isomerized into fructose rather fast, even after 15 minutes but no longer reaction times than 24 hours is not that necessary to investigate.

Table V Summary of relevant literature in this study on isomerization of glucose into fructose with different solid and soluble base catalysts in water solution.

Catalyst	Temperature [°C]	Time [h]	Glucose conc.	Catalyst loading	pH [-]	Glucose conv. [%]	Fructose Yield [%]	Ref.
NaOH	90	24	10 wt-%	1 M	7.5	20	14	(Delidovich and Palkovits, 2014)
NaOH+*p*-tolyl boric acid	50	4.2	23.4 wt-%	1:1 molar ratio of catalyst to glucose	12	-	56	(Barker et al., 1975)
$NaAlO_2$	55	3	40 wt-%	32 wt-%	-	59	41	(Despax et al., 2013)
TEA	100	0.25	0.52 M	12 mol-%	11	59	27	(Carraher et al., 2015)
Hydroxide resin	35	40	1.1 M	-	-	38	32	(Langlois and Larson 1956)
D290 resin	60	12	20 wt-%	10 ml	8	44	25	(Lv et al., 2015)
CaA zeolites	95	-	10 wt-%	2 wt-%	-	11	5.6	(Moreau et al., 2000)
NaA zeolites	95	-	10 wt-%	2 wt-%	-	26	19	(Moreau et al., 2000)

3 CHROMATOGRAPHIC SEPARATION OF FRUCTOSE FROM GLUCOSE

Chromatographic separation methods utilizing ion-exchange resins are usually relatively fast, require less energy, and generate less waste streams compared to some other available separation methods, such as distillation, crystallization, and solvent extraction. Ion-exchange chromatography is especially often used method in the sugar industry to produce concentrated HFCS due to its chemical inertness, capacity ability and selectiveness. (Luz et al., 2008)

3.1 Production of concentrated high fructose corn syrup

Glucose isomerase has not been capable to produce HFCS containing fructose greater than 42 % of the total solids in commercial scale. For some uses, as in the production of acidic soft drinks, FCS-55 (55 % of fructose and 45 % of glucose) is needed to give the same level of sweetness as sucrose. (Birch et al., 2012) Mainly three categories of fructose concentrations of 90 %, 55 % and 42 % in HFCS are produced. HFCS-90 is primarily used as a blender with HFCS-42 to produce HFCS-55. (Parker et al., 2010) This higher fructose content of 90 % has been achieved by the chromatographic separation of fructose from other components of a syrup. A typical flow sheet for the production of 55 % and 90 % of fructose containing HFCS is presented in Figure 22 where chromatographic separation is used to produce HFCS-90 and HFCS-55 is gained from mixing HFCS-42 and HFCS-90 with each other in the right proportion. (Birch et al., 2012) Chromatographic separation of fructose and glucose is carried out industrially commonly using a strong acid cation exchange (SAC) resin and performed continuously under simulated moving bed (SMB) principle (Strube et al. 1998, Dow 2010).

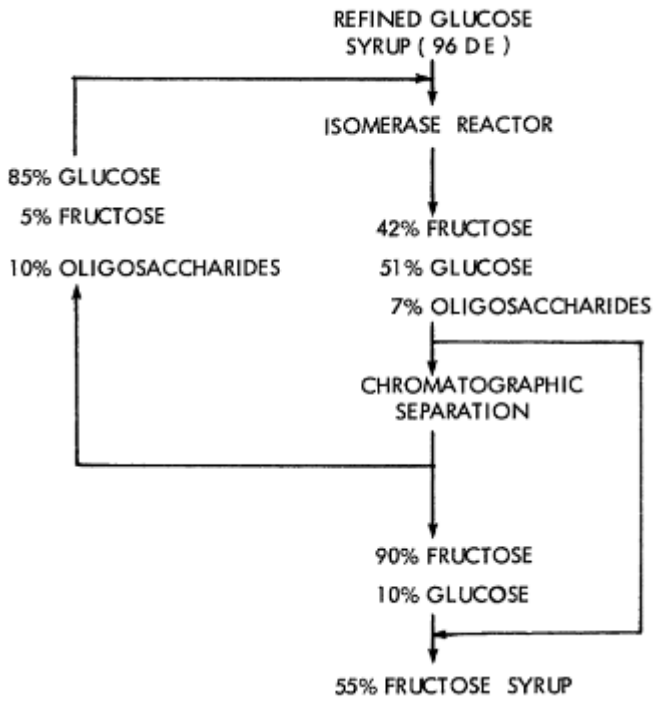

Figure 22 A general flow sheet for the production of 55 % and 90 % fructose containing HFCS (Birch et al., 2012).

3.2 Working principle

Chromatography is a separation method where dissolved components, usually in an aqueous form are separated from each other. The chromatographic separation system includes a stationary phase which is the ion-exchange resin and the moving phase which is the glucose-fructose mixture in the case of HFCS. A feed solution is passed through the stationary phase and different mechanisms and interactions between the stationary phase and the components in the feed leads to the separation of the different components from each other. Although the ion exchange resins are used for the sugar separation, ions are not supposed to be exchanged in the separation process. The most important separation mechanisms of chromatographic separation are size exclusion, ion exclusion and complex formation. Size exclusion refers to separation of components based on their molecule size. Larger size molecules are not able to enter the resin beads and are therefore excluded due to their size. Complex formation refers to the stability difference of complexes formed between the resin counter ion and a component in the feed. For example, some components in the feed are able to form a complex and some components are not, thus explaining the separation. (Al Eid, 2006)

Figure 23 presents graphically three different fractions coming out from the column as a function of time. The y-axis represents the concentration of fructose and glucose. The collected fractions from the column are divided to three categories of a glucose-rich fraction (I), a return fraction (II), and a fructose-rich fraction (III). (Al Eid, 2006)

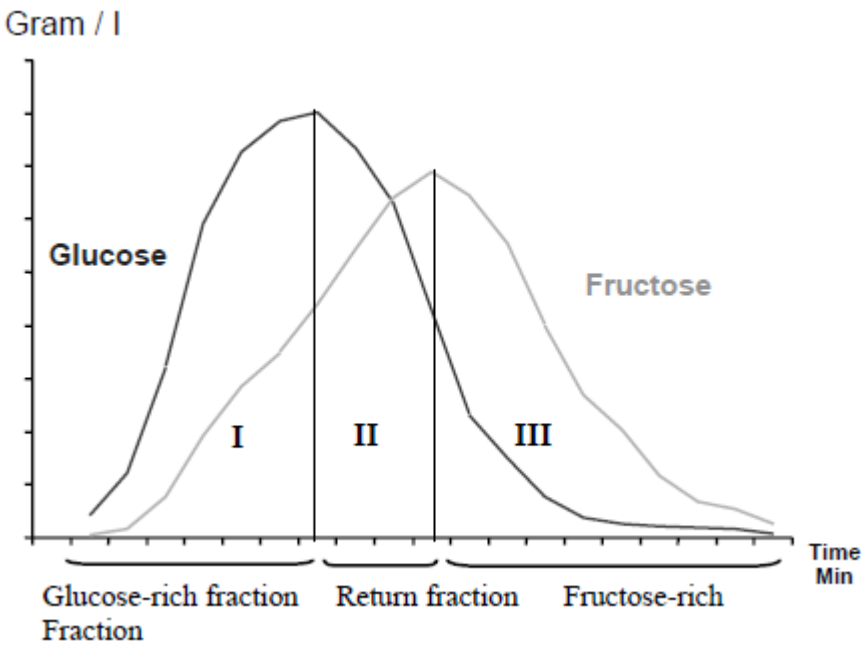

Figure 23 Three different fractions coming out of the chromatographic separation column are glucose-rich-, return- and fructose rich-fractions (Al Eid, 2006).

The glucose has less interaction with the resin and then spends less time in the column and comes out more rapidly than fructose which have stronger interactions with the resin. (DOW, 2017)

3.3 Resin type

The type of resin and its properties in the chromatographic separation column must be carefully designed to get as good as possible separation rate between glucose and fructose. One of the most applied resin used in glucose-fructose chromatographic separation is carried out commonly using a strong acidic cation (SAC) resin in a calcium form. (Viard and Lameloise 1992) Although, the most applied resin for glucose-fructose separations is SAC resin, adsorption equilibria of several monosaccharides as fructose and glucose were evaluated on a weak acid cation (WAC) resin. (Saari et al., 2010)

3.3.1 SAC resin

The glucose-fructose separation with SAC resin in a calcium form is based on fructose forming a complex with Ca^{2+} counter-ion in the resin while glucose forms weaker or no complex with calcium. Glucose is then coming out of the column more rapidly than fructose. (Pedruzzi et al., 2008; Paillat et al. 2000; Al Eid, 2006) Operation temperature of the separation is usually between 60–70 °C which is required for high selectivity (Wang et al., 2016). According to Viard and Lameloise (1992) separation temperature does not affect to the glucose equilibrium and glucose is most likely not adsorbed to the resin that much but only penetrates the pores. While partition coefficient of fructose is dependent of a separation temperature. Lower retention of fructose can be achieved with higher separation temperature in the chromatographic column. (Viard and Lameloise 1992)

Vente et al. (2005) have investigated SAC exchange resin with different cation loadings, such as Na^+, K^+, or Ca^{2+}, for the separation of several monosaccharides from oligosaccharide mixtures under typical industrially applied process conditions at a temperature of 60 °C and high sugar concentrations varying between 0−400 g/l. K^+ and Na^+ and Ca^{2+} loaded resins were compared based on gained sorption isotherms of each sugar. Sorption isotherms describe the interaction between monosaccharide and ion-exchange resin. Figure 24 present the gained isotherm data to glucose, fructose, sucrose, and lactose for the SAC (Dowex 50WX4-400) resin in the Na^+, K^+, and Ca^{2+} forms. Fructose shows notably higher sorption than glucose with Ca^{2+} form resin. However, sorption isotherms of fructose and glucose have only a slight difference using resins loaded with Na^+ and K^+ cations. Fructose forms a complex with a Ca^{2+} ion which explains that fructose sorbed better than glucose in resins. Authors proposed that this may be caused by the differences in the steric orientation of the hydroxyl groups in the monosaccharide molecules. (Vente et al., 2005)

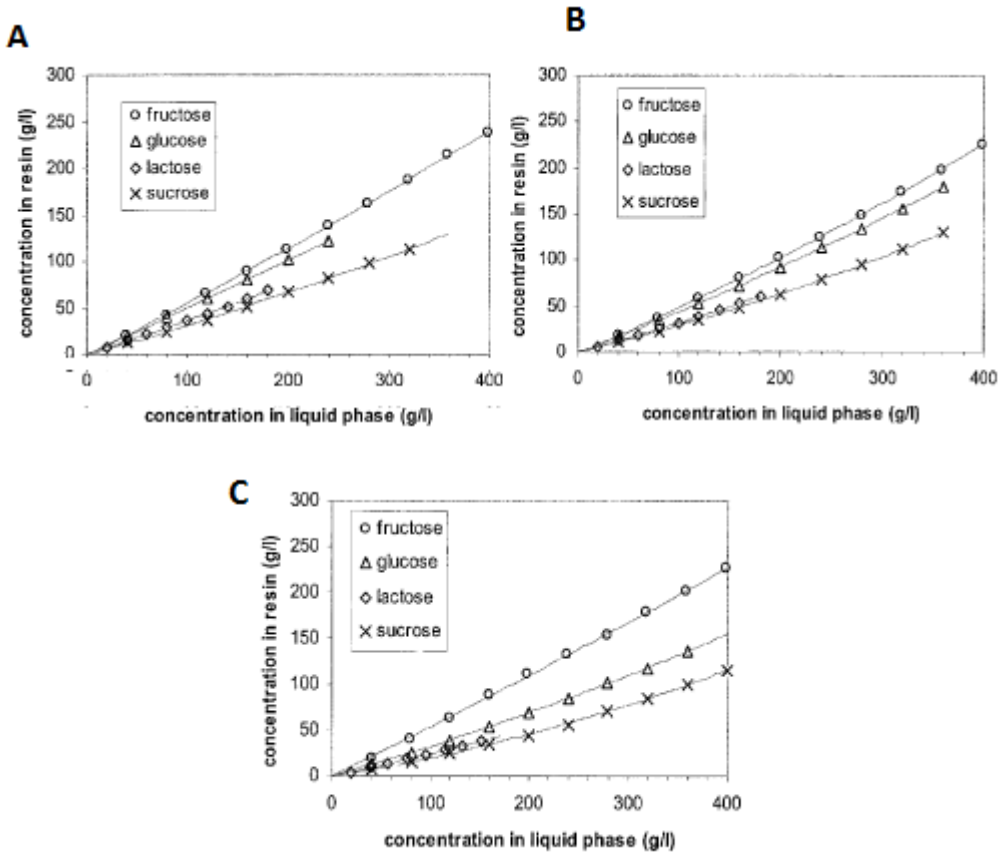

Figure 24 Isotherms for SAC resin (Dowex 50WX4-400) in A) K^+-, B) Na^+-, and C) Ca^{2+}-form at 60 °C (Vente et al., 2005).

Based on these experimental results, the chromatographic separation of fructose and glucose would be better carried out using the calcium loaded SAC exchange resin rather than potassium or sodium loaded resin due to a higher selectivity of calcium loaded resin. From the single sugar isotherms calculated selectivity for glucose-fructose separation was 1,1 for the Na^+ and K^+ ion and even 1,6 for the Ca^{2+} ion. (Vente et al., 2005)

Composition of the feed stream and reaction conditions have to be carefully adjusted and optimized in order to maximize a separation rate and an operational life of the resin. Metal content should be minimized in the feed stream by softening or desalting if necessary. For example, sodium, magnesium and iron can exchange some of the calcium ions from the resin which leads to reduction in the separating capability. (Sigma-Aldrich, 1997; DOW, 2017; Vente et al., 2005) In addition, glucose, the major ingredient of starch sugar, is unstable in alkaline conditions especially combined with higher temperatures and therefore it is important to keep the pH in the proper range and carefully under control. Unstable glucose easily decomposes into acidic or colored compounds. Additionally, glucose might even

convert into fructose, which is known to be even more unstable under hot alkaline conditions. (Sigma-Aldrich, 1997; DOW, 2017)

3.3.2 WAC resin

Overall, treatment of monosaccharides and especially glucose with weak acid cation (WAC) resin has not been under the research as much as treatment with SAC resin. However, some knowledge of glucose interaction with WAC resin can be obtained from a research where Saari et al. (2010) investigated adsorption equilibria for several saccharides, including glucose and fructose, on four types of ion-exchange resins such as earlier introduced WAC and SAC resins. The two other resins were strong base anion (SBA) and weak base anion (WBA) exchange resins. The resins were in their typical ionic form, WAC and SAC resins were sodium-loaded and SBA and WBA resins were sulfate-loaded. (Saari et al., 2010)

Unlike ion-exchange resins with the strong functionality, weak functionality resins are not able to function as ion-exchange resins under the whole pH scale. Therefore, pH of the sugar solutions was adjusted to a suitable level to prevent the ion form reconversion of weak functionality resins. For example, pH was adjusted to 9 with NaOH in the experiments where WAC exchange resin was used. All the experiments were carried out adding a dry resin and a sugar solution (0−350 g/l) to the reaction tubes. The reaction tubes were loaded such that the resin to solution ratio was approximately 2:3. The experiments were carried out at 65 °C for 8 hours. (Saari et al., 2010)

WAC exchange resin require reaction conditions which are challenging for many saccharides. As mentioned earlier, hot alkaline conditions, for example pH 9 and temperature 65 °C used in this experiment, causes the sugars to decompose. From all the sugars investigated, rhamnose and mannose were quite stable because less than 5 % degraded or isomerized during the experiment. However, from 10−30 % of the other sugars, such as glucose and fructose, were isomerized or degraded to other components than investigated sugar. Because the components which were a result from decomposition of the starting sugar had different distribution values, the distribution coefficients could not be determined. It can be concluded that the sodium loaded WAC resin is not suitable for sugar separations because most sugars are unstable and easily isomerized and degraded under alkaline conditions especially combined with elevating temperature. Experiments with SAC resin were

performed under neutral pH and at the same temperature of 65 °C. After 8 hours of reaction, there was not detected degradation or isomerization products of monosaccharides. (Saari et al., 2010)

EXPERIMENTAL PART

4 EXPERIMENTS

Temperature, pH and reaction time are typical variables in the chemical process industry. Based on literature findings, glucose isomerization into fructose and also stability of glucose and fructose can be easily affected by these variables. In addition, literature survey revealed that some metals are able to form complexes between fructose or glucose which can shift the reaction equilibria and catalyze formation of fructose or acidic compounds. Solutions processed in chemical industry may contain varying amounts of metals due to raw materials utilized or chemical additions. In this experimental part, effect of pH, temperature and reaction time on glucose isomerization and degradation reactions is investigated. In addition, catalytic effect of metal loaded WAC resins and addition of metal chlorides on fructose formation is investigated.

4.1 Materials and methods

Five different glucose solutions were investigated; a pure glucose solution and four different industrial lignocellulosic sugar solutions which are named as glucose solution (GS) 1, 2, 3 and 4 from now on. The pure glucose solution was prepared by weighing 250 g of glucose, which was purchased from VWR, and adding distilled water until volume of the solution was one liter. However, the pure glucose solution turned out to be commonly about 280−300 g/l. GS 1−4 contained varying amounts of other monosaccharides besides glucose, such as mannose and xylose, and also carboxylic acids, furan compounds and lignin. Detailed composition of GS 1−4 is shown in Appendix I.

Experimented Na- and Na/Ca/Mg-loaded WAC resins (Finex CA16GC) were delivered by Finex Oy. These WAC resins had the same structure but one resin was loaded with Na and other was loaded with a combination of Na, Ca, and Mg where 45 % was in a calcium form

and 22 % was in a magnesium form. The Na-loaded resin was washed with distilled water for three times. After the resin was washed, pH of washing water was measured to 10,6. The Na/Ca/Mg-loaded resin was washed with distilled water only for once at the beginning. After the resin was washed, pH of washing water was measured to 8,6. Initial pH was adjusted before a resin was added.

Experiments where catalytic effect of metals was investigated, calcium (Ca^{2+}) was added to the pure glucose solution as a calcium chloride ($CaCl_2$), magnesium (Mg^{2+}) as a magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), and sodium (Na^+) as a sodium chloride (NaCl). All three metal chlorides were purchased from VWR. The pure glucose solution was prepared for 0,6 l and each metal chloride was added for a specific amount so that the content of metal chloride in the solution was 0,2 mol/l, i.e. $CaCl_2$ was added for 13,3 g, $MgCl_2 \cdot 6H_2O$ for 24,40 g and NaCl for 7 g. Initial pH was adjusted after the metal addition.

The identification and quantification of carboxylic acids and furans was conducted by HPLC-PDA (high-performance liquid chromatography photodiode array detection method) and analysis of carbohydrates was performed by HPAE-PAD (high performance anion exchange chromatography with pulsed amperometric detection). Quantity analysis of metals were performed by microwave assisted digestion followed by ICP (inductively coupled plasma). pH was measured by Mettler Toledo MP 220 pH meter.

All experiments were carried out by rotating a reaction tubes (total volume 45 ml) inside of an incubator which was set to a desired temperature. Except, samples which were carried out at the room temperature (23 °C), was shaken only manually every 15 minutes instead of continuous rotation inside the incubator. Volume of glucose solution in each reaction tube was 25 ml. Except when glucose solution was studied in presence of WAC resins, each reaction tube was loaded containing 20 g of glucose solution and 20 g of wet resin. One reaction tube was presenting one trial point. For example, if pH range was 6−12 and reaction time was 20 min, 2 h, 6 h and 24 h but reaction temperature was constant, 24 reaction tubes in total was needed which all had a different combination of pH and reaction time. Initial pH was adjusted with 10 w-% or 50 w-% sodium hydroxide (NaOH).

Fructose formation is presented as fructose fraction and fructose yield. Concentration data was converted to fructose fractions with equation (1)

$$\text{Fructose fraction} = \frac{\text{Concentration of fructose (g/l)}}{\text{Total concentration of glucose and fructose (g/l)}} \quad (1)$$

Fructose yield was calculated with equation (2)

$$\text{Fructose yield (\%)} = \text{Glucose conversion} \times \text{Fructose selectivity} \times 100\ \% \quad (2)$$

Where glucose conversion and fructose selectivity were calculated utilizing equations (3) and (4)

$$\text{Glucose conversion} = \frac{\text{Glucose consumed (g/l)}}{\text{Glucose added to the system (g/l)}} \quad (3)$$

$$\text{Fructose selectivity} = \frac{\text{Fructose formed (g/l)}}{\text{Glucose consumed (g/l)}} \quad (4)$$

4.2 Results

4.2.1 Effect of pH and reaction time on isomerization of glucose

Based on literature review, longer reaction time and more alkaline conditions promote fructose formation. Overall, as mentioned in section 2.4, it seems to be worth exploring at least pH range between 8−11 and longer reaction times than 24 hours are not necessary to investigate. Effect of pH and reaction time was investigated at constant temperature of 60 °C. Reaction time was 20 min, 2 h, 6 h and 24 h and initial pH varied between 6−12. Overall, 24 test tubes of different combinations of pH and reaction time was prepared. Three different glucose solutions were tested; the pure glucose solution, GS 1 and GS 2. Concentration of the pure glucose was approximately 280 g/l in this experiment.

The pure glucose solution

Figure 25 shows evolution of fructose fraction and yield as a function of time with varying initial pH. It can be seen that in samples where pH was adjusted to 6,6 and 8,1 fructose was not detected. Glucose started to isomerize into fructose at samples which initial was pH 9 or higher. Delidovich and Palkovits (2014), introduced in section 2.2.1, managed to reach fructose yield of 14 % when initial pH of glucose solution was only 7,5. However, reaction temperature in their experiment was 30 °C higher than reaction temperature selected in this

study which may have caused glucose isomerization into fructose even though the pH was lower.

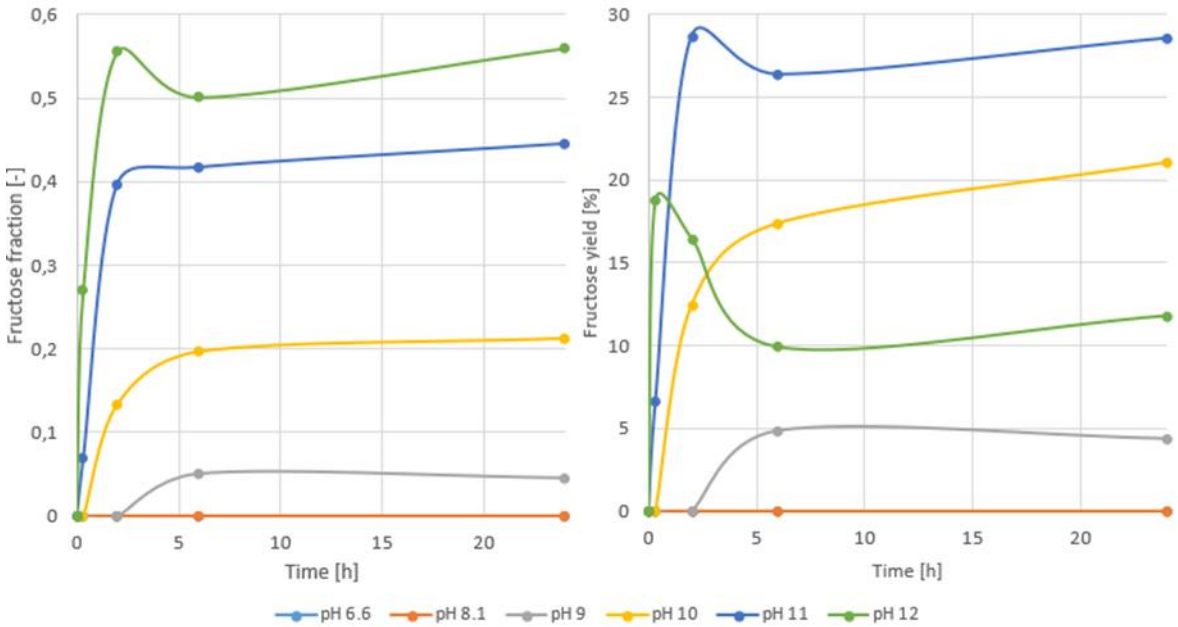

Figure 25 Fructose fraction (left) and yield (right) in the pure glucose solution. Reaction time 20 min−24 h, initial glucose concentration 280 g/l, initial pH 6,6−12, reaction temperature 60 °C.

Glucose began to isomerize into fructose at initial pH 9. Generally, fructose fraction increases with higher initial pH and reaction time. Similar phenomenon was observed in the experiment conducted by Bruijn et al. (1987), introduced in section 2.2.1., where reaction rate of glucose isomerization into fructose increased with higher HO^- content. The highest fructose fraction of 0,56 was detected from a sample which initial pH was 12 already after two hours but fructose yield decreased from 19 to 12 % after 24 hours reaction time due to degradation.

Also, fructose yield increased with higher initial pH, except when initial pH was 12. The highest fructose yield of 29 % was detected from a sample which initial pH was 11 and reaction time was two hours. When initial pH was 12, the highest fructose yield of only 19 % was detected when reaction time was 20 minutes which then decreased during 6 hours to 10 %. Initial pH as high as 12 seems to favor glucose and/or fructose degradation reaction more than glucose isomerization. Glucose and/or fructose seem to degrade only above initial pH 10 since both fructose fraction and yield are still the same, about 20 %, at initial pH 10 after 24 hours.

pH was measured from every sample after experiment and listed in Table VI. Reaction time had a little or no effect on fructose fraction after 6 hours which could be explained by the fact that pH values of most samples after 6 and 24 hours was measured to approximately between 6−7. After 20 minutes there is hardly no change between pH values measured before and after the experiments. Based on literature, neutral pH conditions do not promote glucose isomerization into fructose at least as efficiently as alkaline conditions which could explain why glucose is not isomerizing into fructose after 6 hours. Also, glucose and fructose are known to be unstable under alkaline conditions which could explain poor fructose yield when initial pH of the solution was 12.

Table VI Measured pH values of the pure glucose solution. Reaction time 20 min−24 h, initial glucose concentration 280 g/l, initial pH 6,6−12, reaction temperature 60 °C.

Initial pH	6,6	8,1	9	10	11	12
pH (20 min)	7	7,1	8,9	10,2	11,1	12,1
pH (2 h)	8,9	8,1	8,7	9,7	10,4	10,8
pH (6 h)	7,6	6,6	6,9	6,8	7,2	8,4
pH (24 h)	6,6	6,1	6,5	5,8	6,7	7,7

Figure 26 shows total concentration of glucose and fructose with a function of time with different starting pH values. Concentration of glucose and fructose was observed to decrease over time and with higher initial pH. However, after 6 hours there is a little or no change in the total concentration and solutions seems to have reached their equilibrium state. Fructose is no longer significantly formed after 6 hours and pH was measured to be around 6−7. For example, after 6 hours, the total concentration has dropped down to 57 g/l from 250 g/l when initial pH was 12, but after 24 hours the concentration was 61 g/l. In conclusion, there is no isomerization or degradation when pH of the solution is neutral or below at 60 °C.

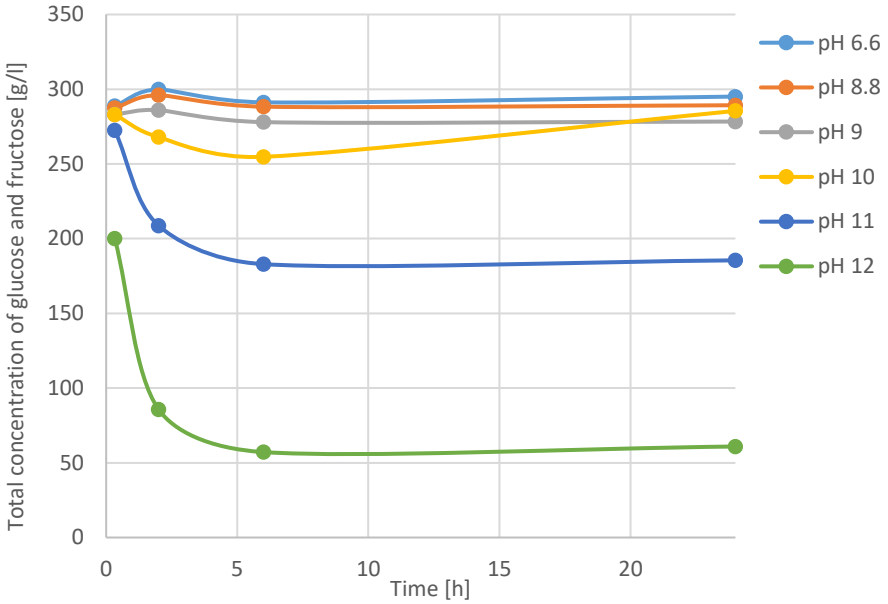

Figure 26 Effect of reaction time and pH on degradation of glucose and fructose in the pure glucose solution. Reaction time 20 min−24 h, initial glucose concentration 280 g/l, initial pH 6,6−12, reaction temperature 60 °C.

Based on literature, decreasing total concentration of glucose and fructose could be due to glucose and/or fructose decomposition into acidic compounds which is also promoted by alkaline conditions. Also, pH decreasing over time refers to this phenomenon. Carboxylic acids and furans were analyzed from samples which reaction time was 6 hours. Figure 27 shows concentration of carboxylic acids as a function of initial pH. Carboxylic acids were detected in samples which initial pH was pH 10 or higher. Total amount of carboxylic acids consisted mainly of succinic acid, formic acid and acetic acid. The highest carboxylic acid concentration of 15 g/l was detected in a sample which initial pH was 12. Any furan compounds, such as HMF, was not detected in any sample.

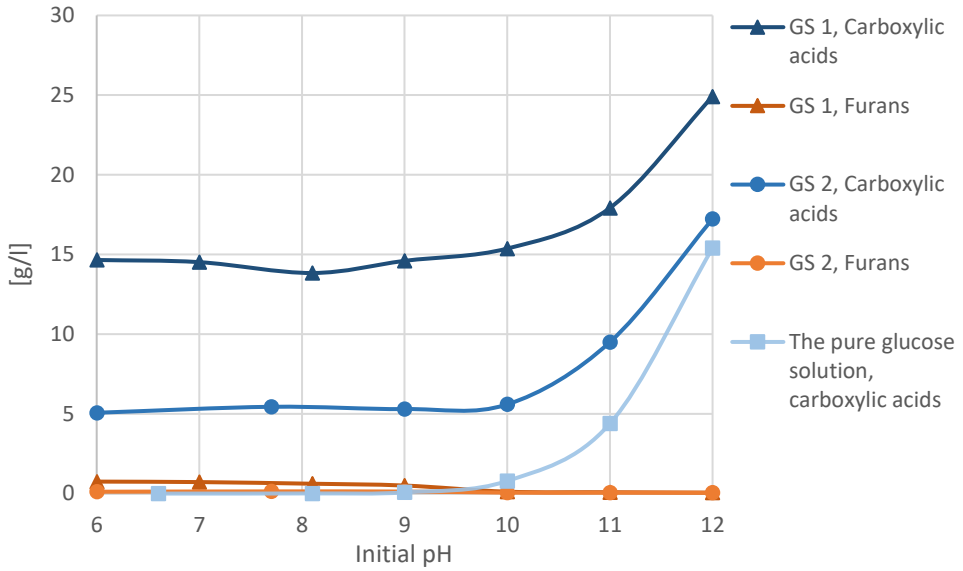

Figure 27 Effect of initial pH on formation of organic acids in the pure glucose solution (280 g/l), GS 1 (250 g/l) and GS 2. Reaction time 6 h, initial pH 6−12, initial glucose concentration 280 g/l (the pure glucose solution), 272 g/l (GS 1) and 290 g/l (GS 2), temperature 60 °C.

All of unknown compounds was not able to detect with the analysis of carboxylic acids and furans. A sample which initial pH was 12 and reaction time 24 h was further investigated and hydroxy acid compounds was analyzed. Variety of hydroxy acids was identified which had the same carbon skeleton of glucose and fructose but also those which had shorter carbon chains. Similar acidic compounds were identified than in the study by Yang and Montgomery (1996), in section 2.2.2, where acidic compound formation under alkaline conditions was investigated. Although all the compounds in the sample were not identified, further investigations showed that total amount of carbon did not change during the experiment.

Figure 28a shows 6 reaction tubes of the pure glucose solution with varying starting pH values from 6,6 to 12 after 6 hours. The glucose solution was discolored only in the tubes which initial pH was between 10 and 12 and the solution was darker on tubes which had a higher initial pH. Also, carboxylic acids were only detected in samples which were discolored.

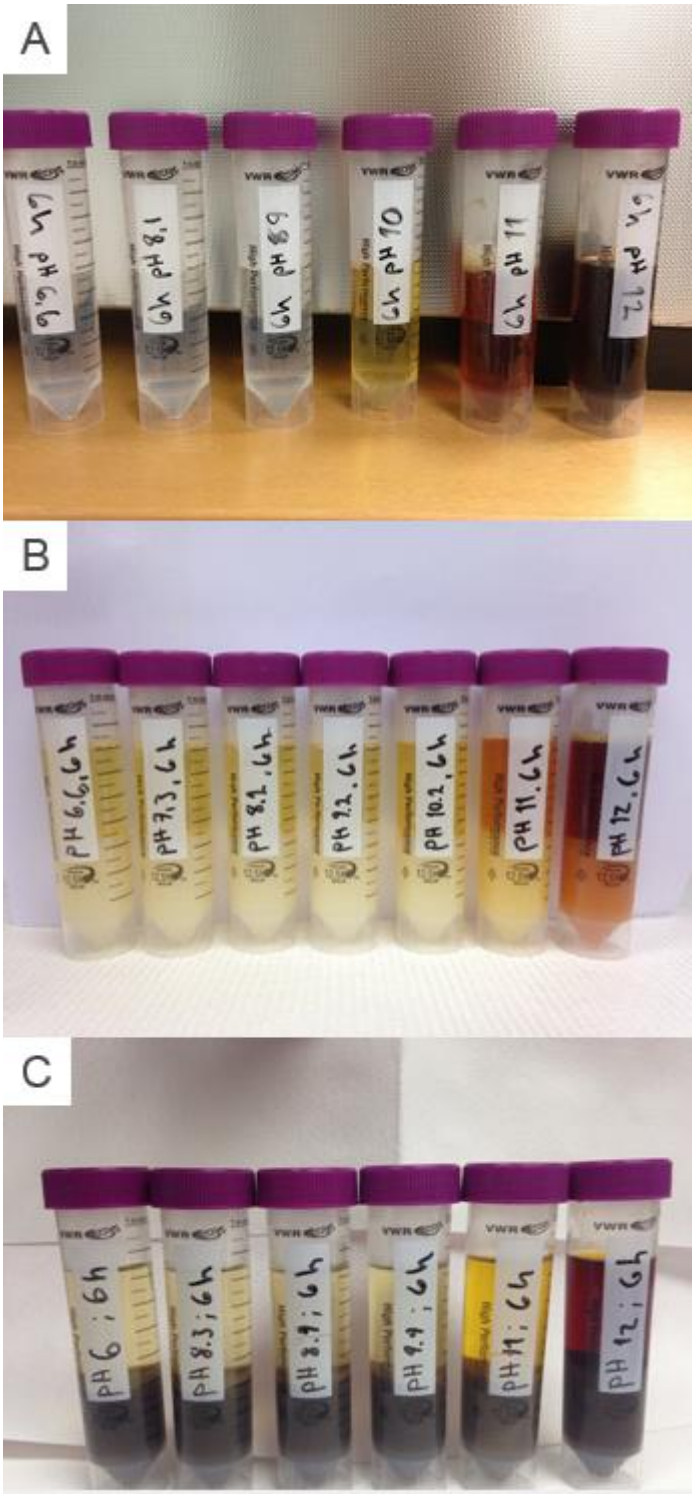

Figure 28 The pure glucose solution was discolored during experiments. The color of the solution was darker when initial pH was higher. The pure glucose solution A) without the resin, initial pH 6,6−12; B) in presence of Na-loaded WAC resin, pH before resin addition 6,6−12; C) in presence of Na/Ca/Mg-loaded WAC resin, pH before resin addition 6−12. Reaction time 6 h and temperature 60 °C.

It may be possible that fructose is more unstable than glucose under the selected process conditions and therefore fructose yield decrease when fructose fraction increase. However,

glucose degradation (into acidic compounds) reaction rate constants were always about five times higher than fructose degradation reaction rate constants in the experiment by Bruijn et al. (1987), introduced in section 2.2.1, where degradation reaction rate constants of glucose and fructose were determined with different hydroxide ion concentration. However, reaction temperature was about 20 °C higher in experiments carried out by Bruijn et al. (1987) than reaction temperature selected in this study which may have effect on stability of glucose and fructose.

Glucose solution 1

Initial pH of GS 1 was adjusted to 6−12. Only samples which initial pH was 8,1; 10 and 12 were carried out for 24 hours. Evolution of fructose fraction and yield is presented as a function of time in Figure 29.

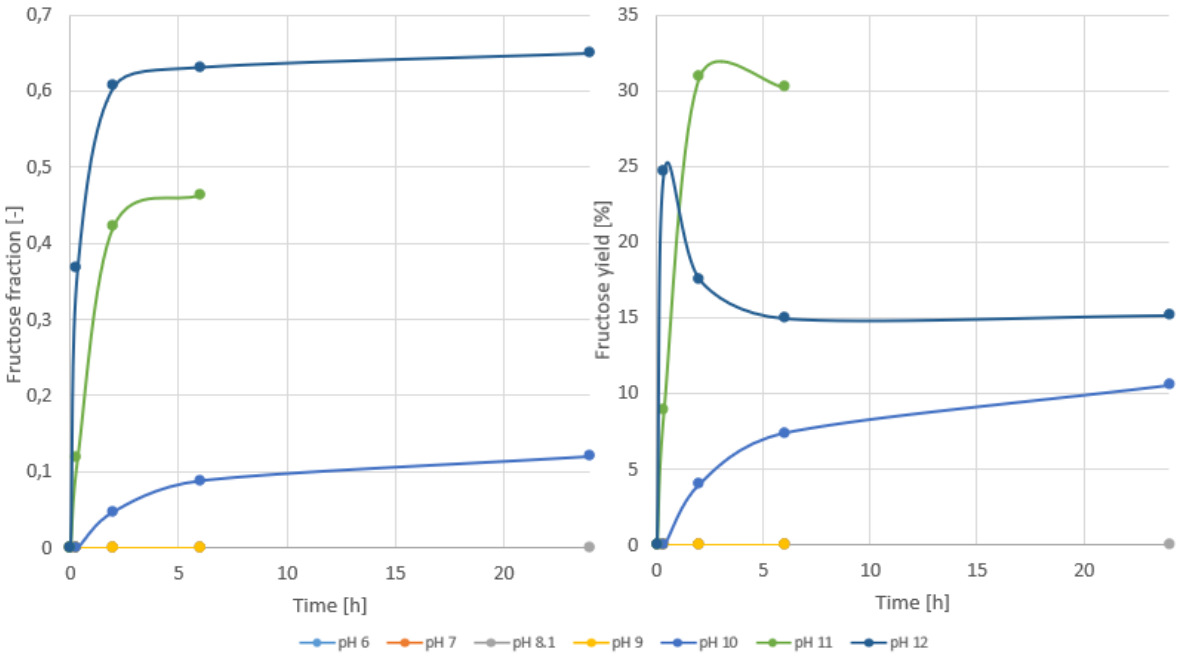

Figure 29 Fructose fraction (left) and yield (right) in GS 1. Reaction time 20 min−24 h, initial glucose concentration 272 g/l, initial pH 6−12, reaction temperature 60 °C. Only solutions which initial pH was 8,1; 10,2 and 12 were allowed to react for 24 hours.

The highest fructose fraction of 0,66 was detected in a sample which initial pH was 12 and reaction time was 24 h but fructose yield was only 15 %. A solution which initial pH was 11 gives a higher fructose yields than initial pH 12, as in the experiment with the pure glucose solution. The highest fructose yield of 31 % was reached after two hours of reaction when initial pH was 11. In addition, fructose yield begins to decrease after 20 minutes in the

solution which initial pH was 12 and after 2 hours in the solution which initial pH was 11, similarly as in the experiment with the pure glucose solution.

The same phenomenon is observed as in the experiments with the pure glucose solution, reaction time has only a little effect on fructose fraction after 6 hours. For example, after 6 hours fructose fraction was 0,63 in a trial point which pH was adjusted to 12 at the beginning but after 24 hours fructose fraction was increased only to 0,66. This can be explained again by pH decreasing close to neutral in all glucose solutions during experiment which do not promote glucose isomerization into fructose anymore. Table VII presents measured pH values in every sample after the experiment.

Table VII Measured pH values of GS 1. Reaction time 20 min−24 h, initial glucose concentration 272 g/l, reaction temperature 60 °C. Only solutions which initial pH was 8,1; 10,2 and 12 were allowed to react for 24 hours.

Initial pH	6	7	8,1	9	10	11	12
pH (20 min)	5,8	6,4	6,9	7,3	8,9	11,1	12
pH (2 h)	5,7	6,1	6,5	7	8,3	10,2	10,9
pH (6 h)	5,6	6	6,3	6,8	7,8	8,8	9,5
pH (24 h)			6		7,1		8,4

Difference between experiment with the pure glucose solution and GS 1 is that fructose is not detected until samples which initial pH was 10 and higher while in the experiments with the pure glucose solution fructose was detected even in samples which pH was adjusted to 9. However, GS 1 gives higher fructose fractions and yields in samples which have higher initial pH as 11 and 12. For example, with GS 1, the highest fructose fraction of 0,65 and 15 % yield was detected in a sample where pH was adjusted to 12 at the beginning and reaction time was 24 h while with the pure glucose solution in the same conditions fructose fraction was 0,56 and yield was 12 %. This could be due to pH decreasing less in GS 1 than in the pure glucose solution during the experiment. In the pure glucose solution pH was 8,4 and 7,7 after 6 and 24 hours in samples which starting pH was 12, while in the same conditions with GS 1 pH was 9,5 and 8,4 after 6 and 24 hours. Lower initial glucose content in GS 1 can be the reason for less acidic compounds formed. Also, it may be possible that other compounds in GS 1 are affecting stability of glucose and fructose in GS 1.

Figure 30 present total concentration of glucose and fructose as a function of time in GS 1. A significant decrease in total concentration is observed only in samples which starting pH was 11 and 12. Similarly with fructose fraction, there is a little or no effect on the total concentration after 6 hours. For example, total concentration was 65 g/l after 6 hours and 64 g/l after 24 hours in a sample which initial pH was 12. Total concentration of glucose and fructose seems to change similarly over time and at the same level in GS 1 and in the pure glucose solution. In conclusion, other components in glucose solution, such as organic acids and other saccharides, do not have an effect on glucose isomerization or degradation.

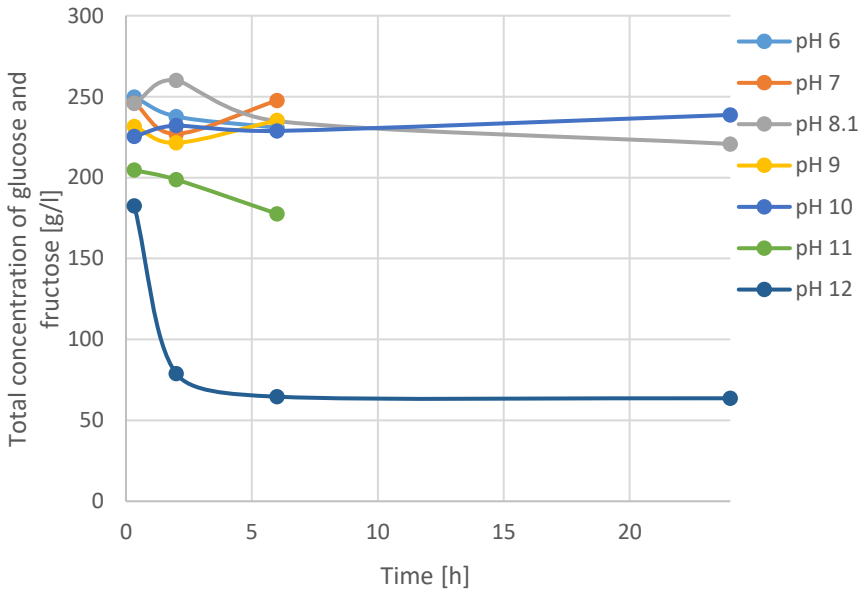

Figure 30 Effect of reaction time and pH on degradation of glucose and fructose in GS 1. Reaction time 20 min−24 h, initial glucose concentration 272 g/l, initial pH 6−12, reaction temperature 60 °C. Only solutions which initial pH was 8,1; 10,2 and 12 were allowed to react for 24 hours.

GS 1 contained carboxylic acids (17,6 g/l) and furans (0,8 g/l) before the experiment. Figure 27 showed total concentration of carboxylic acids and furans as a function of initial pH when reaction time was 6 hours. Increase of concentration of carboxylic acids in GS 1 was about 10 g/l between initial pH 6 and 12 while in the pure glucose solution increase was 15 g/l. At the beginning, carboxylic acids in GS 1 consisted mainly of citric acid, succinic acid and acetic acid. However, in sample which initial pH was 12, after 6 hours of reaction amount of carboxylic acids consisted mainly of formic acid, succinic acid and acetic acid, i.e. there was hardly any citric acid and more formic acid was observed instead.

Glucose solution 2

Fructose fraction and yield is presented in Figure 31 as a function of time with different initial pH values. In general, GS 2 gives a bit higher fructose fractions than GS 1 which is most likely due to GS 2 containing 5 g/l of fructose before the experiment. For example, fructose fraction of 0,18 was detected after 24 hours in GS 2 which pH was adjusted to 10, while at the same conditions fructose fraction of 0,12 was detected in GS 1. The highest fructose fraction of 0,65 was detected in a trial point where initial pH was 12 and reaction time was 24 hours but fructose yield was only 12 %. Profiles of fructose fraction and yield are similar between GS 1 and GS 2. Except, GS 2 which initial pH was 10 and 12 led to the same fructose yield of 12 % after 24 hours, while in GS 1 initial pH 12 resulted notably higher fructose yield than initial pH 10.

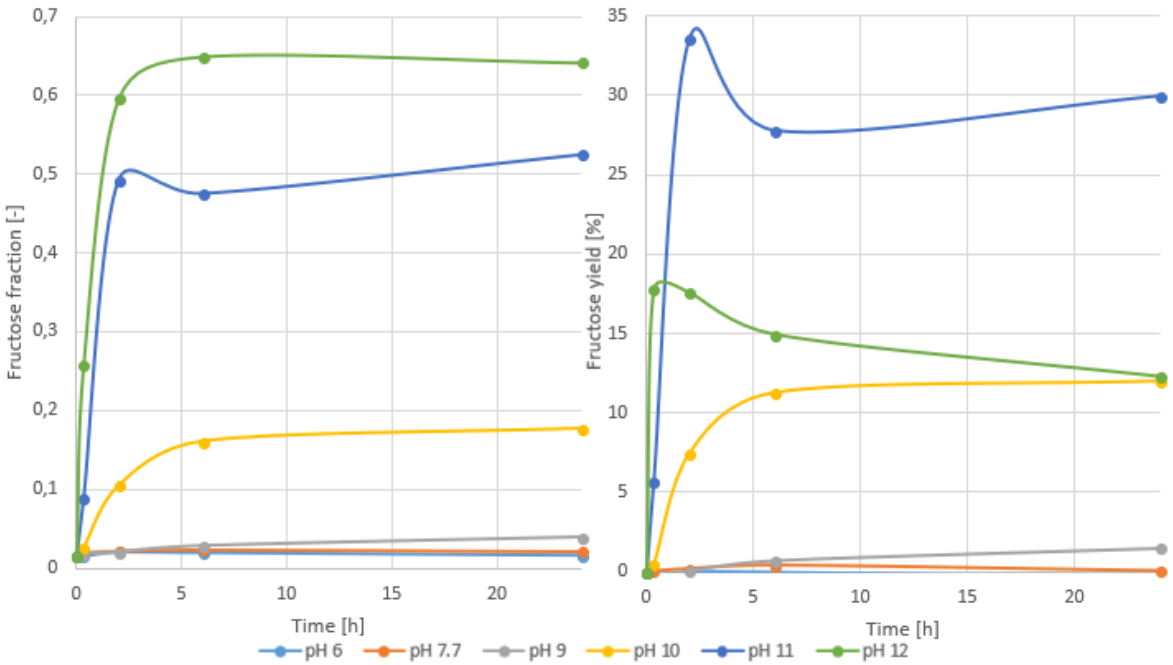

Figure 31 Fructose fraction (left) and yield (right) in GS 2. Reaction time 20 min−24 h, initial concentration of glucose 285 g/l and fructose 5 g/l, initial pH 6−12, reaction temperature 60 °C.

Also, pH decreased similarly in GS 1 and GS 2 during the experiment which is logical since gained fructose fractions and yields were about at the same level in both experiments. Table VIII shows measured pH values in GS 2 after the experiment.

Table VIII Measured pH values of GS 2. Reaction time 20 min−24 h, initial total concentration of glucose 285 g/l and fructose 5 g/l, reaction temperature 60 °C.

Initial pH	6	7,7	9	10	11	12
pH (20 min)	5,9	6,7	7,3	9,1	10,7	11,8
pH (2 h)	5,5	6,1	6,7	8,4	9,8	10,7
pH (6 h)	5,4	5,9	6,6	7	7,7	8,9
pH (24 h)	5,3	5,7	6,2	6,5	6,9	7,9

Total concentration of glucose and fructose is presented in Figure 32 as a function of time with varying initial pH. The total concentration of GS 1 and GS 2 decreased similarly over time and reached the same concentration levels after 24 hours. The total concentration of GS 2 decreased from 290 g/l to 62 g/l after 24 hours in a sample where initial pH was 12. While at the same reaction conditions the total concentration of GS 1 decreased from 272 g/l to 64 g/l.

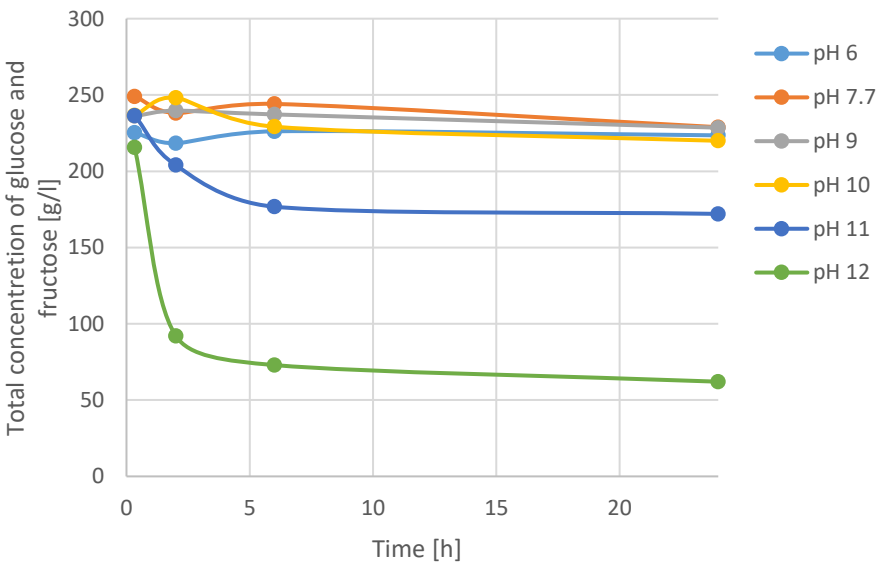

Figure 32 Effect of reaction time and pH on degradation of glucose and fructose in GS 2. Reaction time 20 min−24 h, initial total concentration of glucose 285 g/l and fructose 5 g/l, initial pH 6−12, reaction temperature 60 °C.

Earlier, Figure 27 presented concentration of carboxylic acids and furans in GS 2 as a function of initial pH. Carboxylic acids and furans were analyzed only from samples which reaction time was 6 hours. Similarly, as in the case of GS 1, in GS 2 concentration of carboxylic acids start to increase not until in samples which initial pH was 11 and higher.

Concentration of carboxylic acids increased from 5 g/l to 17 g/l between initial pH 6 and 12 in GS 2 while in GS 1 concentration of carboxylic acids increased from 15 g/l to 25 g/l. GS 2 consisted less carboxylic acids at the beginning but in both solutions carboxylic acid content increased about 10 g/l. Similarly, with GS 1, concentration of carboxylic acids in a sample which initial pH was 12, consisted mainly of formic acid, succinic acid and acetic acid.

It can be concluded that GS 1 and GS 2 acted for the most part similarly as the pure glucose solution. Glucose isomerized and degraded similarly under the same reaction conditions in GS 1, GS 2 and in the pure glucose solution. Regardless of the fact that GS 1 and GS 2 contained varying amounts of impurities as carboxylic acids, other saccharides than glucose and lignin.

4.2.2 Effect of temperature on isomerization of glucose

Based on literature, temperature might have a promoting effect on glucose conversion into fructose. All the previous experiments have been carried out at constant temperature of 60 °C. Effect of temperature on glucose isomerization and degradation was chosen to investigate at 23, 40 and 60 °C with the pure glucose solution and also with GS 3 and GS 4. Glucose content turned out to be approximately 300 g/l in this experiment. Based on earlier experiments, glucose is isomerizing into fructose under pH range between 10−12. However, under pH 10 and 11 glucose and fructose do not degrade into acidic compounds as much as under pH 12. Therefore, initial pH of the pure glucose solutions was chosen to adjust to 10 and 11. Reaction time was 2, 4 and 6 hours. Figure 33 presents effect of reaction temperature varying from 23−60 °C on fructose formation.

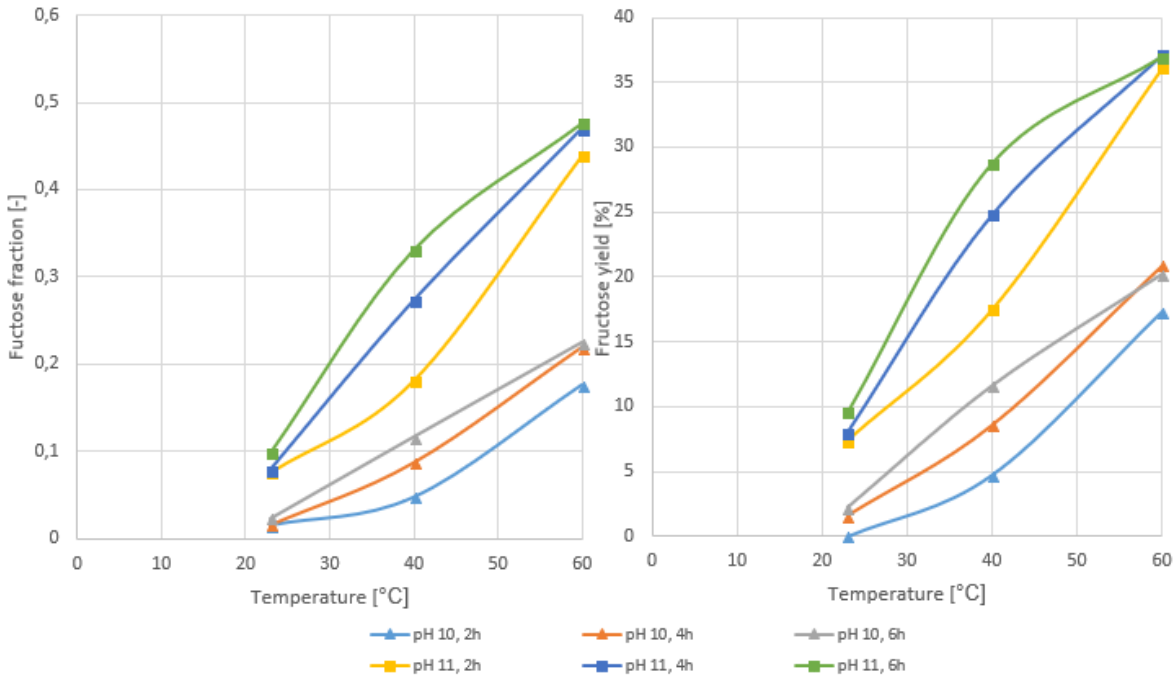

Figure 33 Fructose fraction (left) and yield (right) in the pure glucose solution when reaction temperature was 23, 40 and 60 °C. Reaction time 2−6 h, initial glucose concentration 300 g/l, initial pH 10 (▲) and 11 (■).

The reaction temperature seems to have promoting effect on glucose isomerization into fructose. For example, fructose fraction is around 0,1 in samples which were carried out under 23 °C and initial pH was 11 but between 0,4−0,5 when carried out at 60 °C. Also, fructose yield increases as a function of reaction temperature. Lv et al. (2015) got somewhat different outcome when effect of reaction temperature between 30−60 °C on glucose isomerization was investigated in presence of strongly basic anion exchange resins, introduced in section 2.2.1. Higher reaction temperatures led to higher fructose yields only until 50 °C and slightly decreased when experiments were carried out under 60 °C.

As expected, samples which initial pH was 11 gave significantly higher fructose yields than samples which initial pH was 10. For example, the highest fructose fraction was detected in a trial point where temperature was 60 °C and reaction time 6 h for the both pH series, and under pH 10 the highest fructose fraction was 0,22 and under pH 11 it was 0,48. At the same trial point, fructose yield increased from 20 % to 37 % between initial pH 10 and 11.

Interestingly, reaction time varying from 2 to 6 hours had a significant effect on fructose formation only at 40 °C. Reaction time have no effect at 23 °C on fructose fraction or yield because glucose hardly isomerizes into fructose under such low temperature. However, reaction time have no effect on fructose formation at 60 °C because glucose isomerization

proceeds fast; nearly the same fructose fraction and yield is detected whether reaction time was 2 or 6 hours. While at 40 °C, glucose isomerization proceeds slower because gained fructose fraction and yield increases over time. For example, fructose fraction was 0,18 after 2 hours and increased even to 0,33 after 6 hours when initial pH was 11 at 40 °C.

Unlike in all the previous experiments which were carried out at 60 °C, there was hardly no difference between initial pH and pH after experiments which reaction temperature was 23 or 40 °C. However, at 60 °C after 6 hours, pH of the pure glucose solution had decreased to 7,3 and 8,1 from initial pH 10 and 11, respectively. The same neutralization phenomenon caused by formation of acidic compounds was observed also in earlier experiments with the pure glucose solution at 60 °C. Lower amounts of acidic compounds formed indicates that glucose and fructose are more stable under 23 and 40 °C. Measured pH values are listed in Table IX.

Table IX Measured pH values of the pure glucose solution. Reaction temperature was 23, 40 and 60 °C, time 2−6 h, initial glucose concentration 300 g/l, initial pH 10 and 11.

Temperature [°C]	**23**	**23**	**40**	**40**	**60**	**60**
Initial pH	10	11	10	11	10	11
pH (2 h)	10,3	11,2	10,6	11,4	9,4	10,2
pH (4 h)	10,5	11,4	10,5	11,5	8,4	9,1
pH (6 h)	10,6	11,4	10,6	11,4	7,3	8,1

Evolution of total concentration of glucose and fructose as a function of reaction temperature is presented in Figure 34. Similar decrease on total concentration can be observed as before and higher reaction temperatures clearly accelerate glucose and/or fructose degradation. For example, after 6 hours at 23 °C, total concentration of glucose and fructose was 294 g/l in a solution which initial pH was 11 and dropped down to 233 g/l when reaction temperature was 60 °C. In addition, reaction time varying from 2 to 6 hours had a clear effect on glucose and/or fructose degradation, as on fructose formation, only at 40 °C. Glucose and/or fructose are degraded faster under higher reaction temperature.

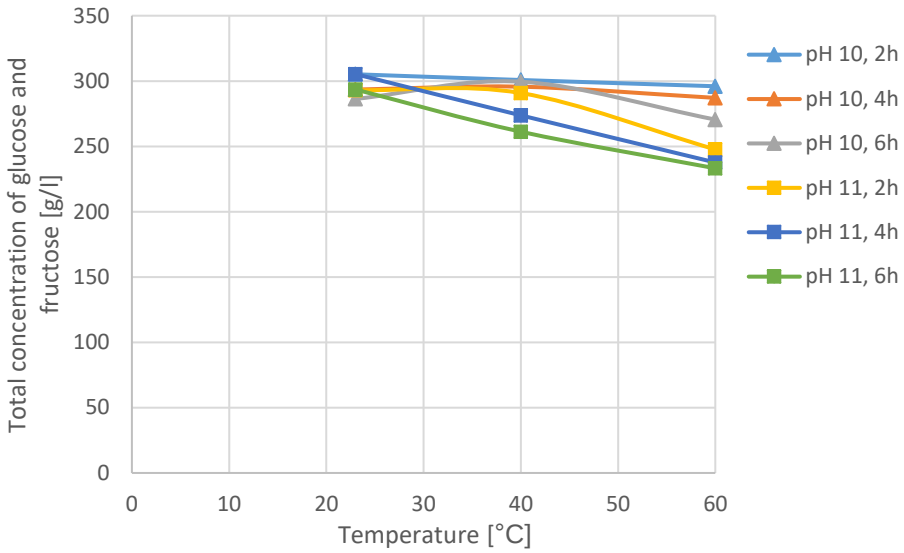

Figure 34 Effect of reaction temperature on degradation of glucose and fructose in the pure glucose solution. Reaction temperature 23, 40 and 60 °C, time 2−6 h, initial glucose concentration 300 g/l, initial pH 10 (▲) and 11 (■).

GS 3 and GS 4 reacted similarly as the pure glucose solution under investigated reaction temperatures. Except total concentration did not decrease almost at all during experiments under tested temperatures. Concentration of glucose solution might have effect on glucose isomerization and degradation reactions. It might be possible that total concentration of GS 3 and 4 did not decrease as much as the total concentration of the pure glucose solution during experiments since the pure glucose solution had significantly higher glucose content (300 g/l) at the beginning than GS 3 (127 g/l) and GS 4 (110 g/l). Fructose fraction, yield and total concentration of glucose and fructose as a function of reaction temperature as well as table of measured pH values after experiment are presented in Appendix III.

4.2.3 Catalytic effect of WAC resins on isomerization of glucose

The pure glucose solution, GS 1 and GS 2 was experimented in presence of WAC resins, one was Na-loaded and other was Na/Ca/Mg-loaded. However, the Na-loaded resin was experimented only with the pure glucose solution. Experiments were carried out at 60 °C, reaction time was 20 min−24 h and initial pH was adjusted to vary between 6−12. Adjustment of initial pH was made before the resin was added to the reaction tubes.

The pure glucose solution with the Na-loaded WAC resin

At first, the pure glucose solution in presence of the Na-loaded WAC resin was investigated. Figure 35 present fructose fraction and yield as a function of time with different initial pH values of the pure glucose solution. Only samples which initial pH was 8,2; 10,2 and 12 reacted for 24 h.

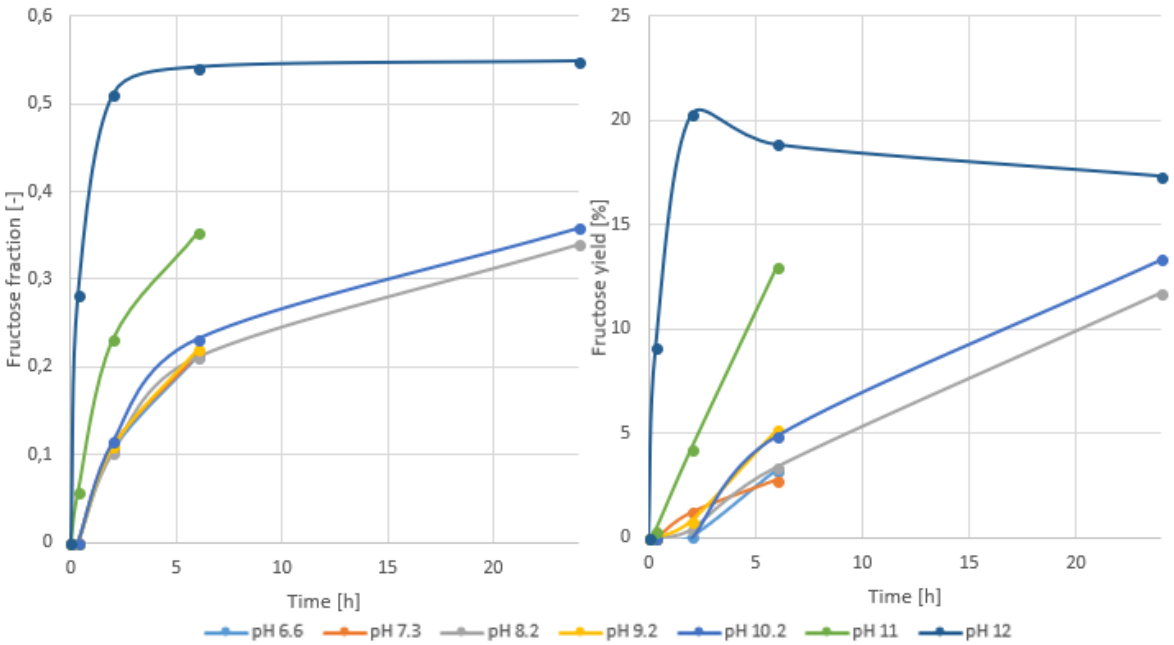

Figure 35 Fructose fraction (left) and yield (right) in the pure glucose solution in presence of the Na-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 6,6−12, reaction temperature 60 °C. Only solutions which initial pH was 8,2; 10,2 and 12 were allowed to react for 24 hours.

Generally, the Na-loaded WAC resin seems to promote fructose formation more than without the resin when initial pH was 6,6−10. Even glucose solutions which initial pH was 6,6−8,2 isomerizes into fructose with the resin, unlike without the resin. However, solutions which initial pH was 11 and 12 led to higher fructose fractions without the resin. The highest fructose fraction of 0,55 was detected in a sample which initial pH was 12 and reaction time was 24 hours but yield of fructose was only 17 %. The highest fructose yield of 20 % was gained when reaction time was two hours and initial pH was 12.

Higher fructose fractions in samples which initial pH was low is due to the fact that initial pH was measured before resin was added. Measured pH values of samples after experiments was higher with than without the resin. Table X shows pH values after 20 min, 2 h, 6 h and 24 h. After 20 minutes and two hours pH increased to 9−11 in all glucose solutions in

presence of the Na-loaded resin, regardless of initial pH. While after 20 minutes and two hours, pH of the pure glucose solution without the resin was hardly changed from the pH which was adjusted at the beginning. It can be concluded that glucose was isomerized into fructose even in samples which initial pH was 6,6−8,2 because pH increased to 9−11 when the resin was added. The real initial pH of the pure glucose solution is most likely very close to measured pH value after 20 minutes. In samples which were allowed to react 6 and 24 hours, pH was acting similarly with and without the resin, i.e. pH was settled between 6 and 8 in all samples.

Table X Measured pH values of the pure glucose solution before and after addition of Na-loaded WAC resin. Reaction time 20 min−24 h, reaction temperature 60 °C. Only solutions which initial pH was 8,2; 10,2 and 12 were allowed to react for 24 hours.

pH before resin addition	6,6	7,3	8,2	9,2	10,2	11	12
pH (20 min)	9,7	9,6	9,6	9,6	9,7	10,3	11,4
pH (2 h)	9,5	9,2	9,4	9,4	9,4	9,9	10,4
pH (6 h)	8	7,8	7,7	7,6	7,6	7,6	7,6
pH (24 h)			7,1		7,1		7,1

Equilibrium reactions between the resin and water is introduced in equation (5), where forward reaction produces NaOH which increases pH of the solution. pH of the pure glucose solution might be increasing because position of equilibrium lies on the right hand side and alkaline conditions promote glucose isomerization into fructose as reported earlier.

$$\text{WAC-COO}^- \text{Na}^+ + \text{H}_2\text{O} \rightleftharpoons \text{WAC-COO}^- \text{H}^+ + \text{Na}^+\text{OH}^- \qquad (5)$$

Similarly, as in the experiments without the resin, total concentration of glucose and fructose was observed to decrease in presence of the resin in samples which had a longer reaction time and higher initial pH. Figure 36 present total concentration of glucose and fructose as a function of time with different initial pH values. Concentration of glucose solution was between 150−200 g/l after 20 minutes although 250 g/l glucose solution was prepared at the beginning. The WAC resin was added after it was washed with distilled water for three times and no drying was done. The water among the resin must have caused some dilution of

glucose solution. Generally, glucose and/or fructose degraded more without the resin especially when taken into account that the glucose solution had higher glucose concentration at the beginning of experiments without resin. For example, after 6 hours total concentration of glucose and fructose was decreased to 75 g/l in a trial point where pH was adjusted to 12 while at the same conditions without the resin total concentration of glucose and fructose was 57 g/l. Concentration of glucose solution might have an effect on both isomerization and degradation reactions.

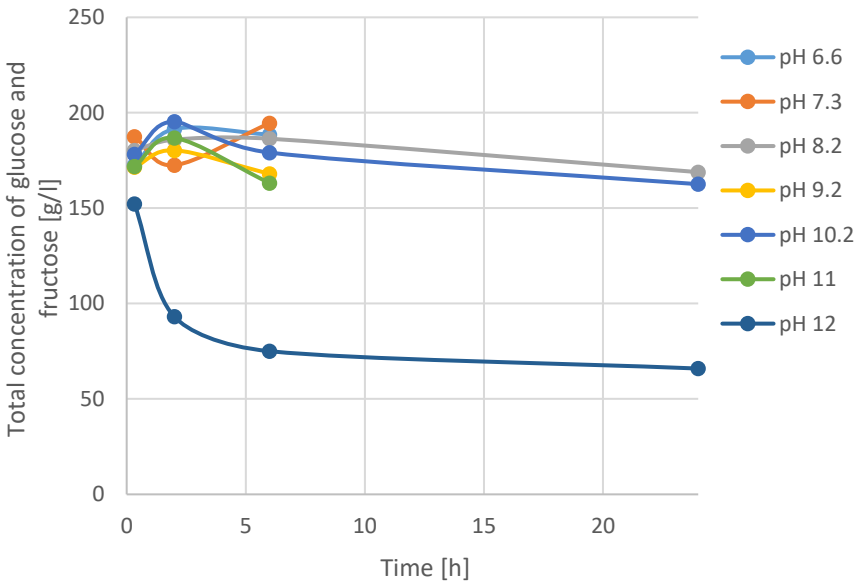

Figure 36 Effect of reaction time and pH on degradation of glucose and fructose in the pure glucose solution in presence of the Na-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 6,6−12, reaction temperature 60 °C. Only solutions which initial pH was 8,2; 10,2 and 12 were allowed to react for 24 hours.

Carboxylic acids and furans were analyzed from samples which reaction time was 6 hours. Figure 27 shows concentration of carboxylic acids as a function of initial pH. Similarly, with and without the resin, furan compounds were not observed. Overall, concentration of carboxylic acids was slightly lower in the samples with the resin than without the resin. This is most likely due to lower initial glucose content of samples in presence of the resin. The highest concentration of carboxylic acids was 6,7 g/l which was detected at a trial point where initial pH was 12 while in the same conditions without the resin the highest concentration of carboxylic acids was 15 g/l. Similarly, in the case without the resin, measured carboxylic acids consisted mainly of succinic acid, formic acid and acetic acid.

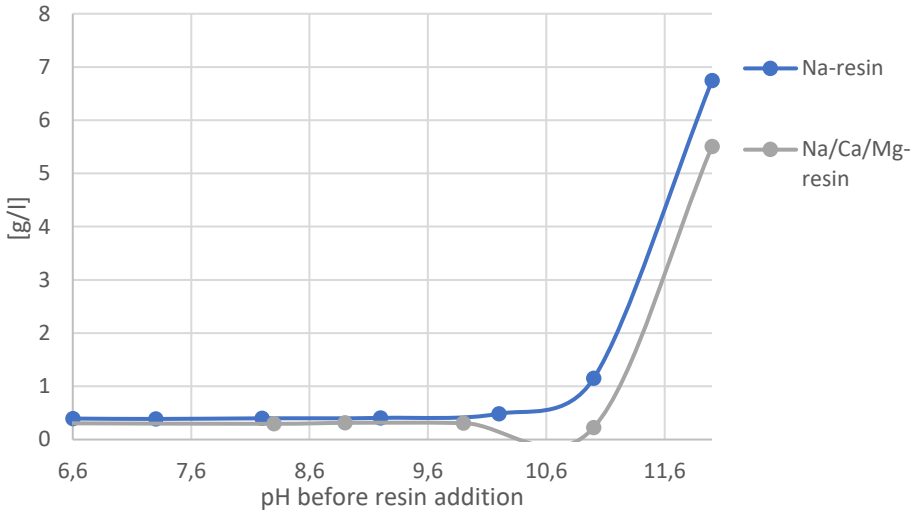

Figure 37 Effect of pH before resin addition on formation of carboxylic acids in the pure glucose in presence of Na- or Na/Ca/Mg-loaded WAC resin. Reaction time 6 h, initial pH 6,6−12, temperature 60 °C.

Figure 28b showed 7 test tubes after 6 hours of reaction which contained the pure glucose solution in presence of the Na-loaded WAC resin and initial pH varied from 6,6 to 12. It can be seen, when comparing Figure 28a and Figure 28b, that all samples were discolored with the WAC resin while without the resin only samples which initial pH was 10 or higher was discolored. Again, this can be due to pH increasing to 9−11 after the resin was added to the pure glucose solution which causes degradation of glucose and fructose.

The pure glucose solution with the Na/Ca/Mg-loaded WAC resin

Experiment with the pure glucose solution and the Na/Ca/Mg-loaded WAC resin was carried out in the same way as with the Na-loaded resin. Figure 38 presents fructose fraction and yield as a function of time with initial pH varying from 6 to 12. Generally, the Na/Ca/Mg-loaded resin gave lower fructose fractions than Na-loaded resin. For example, samples which initial pH was adjusted to vary between 6−9,9 resulted fructose fraction of 0,12 at highest after 24 hours in presence of the Na/Ca/Mg-loaded resin but with the Na-loaded resin samples which initial pH was adjusted to vary between 6,6−10,2 resulted fructose fraction of 0,36 at highest. The highest fructose fraction of 0,53 was detected in a trial point where initial pH was 12 and reaction time 24 h but fructose yield was 14 %. Similarly in the experiment without the resin, samples which initial pH was 11 result higher fructose yields than samples where initial pH was 12, except when reaction time was two hours. The highest fructose yield of 24 % was detected in a trial point where initial pH was 12 and reaction time

2 hours but after 24 hours it was decreased to 14 %. While in a sample which initial pH was 11, fructose yield increased from 15 % to 20 % when reaction time was 2 and 24 hours.

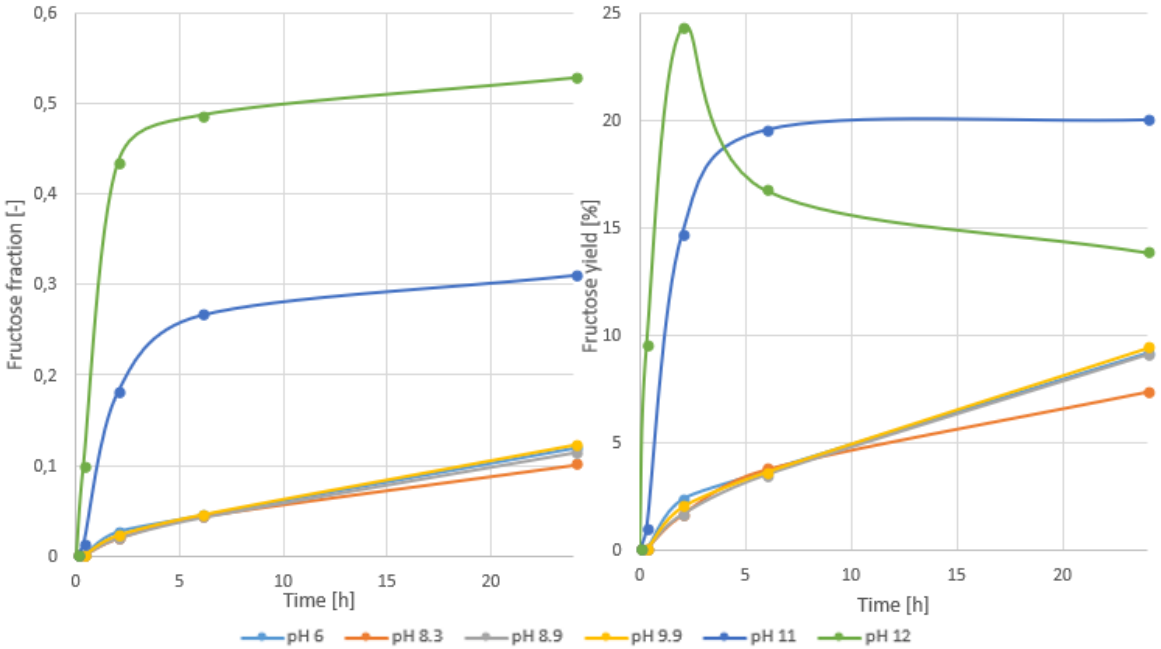

Figure 38 Fructose fraction (left) and yield (right) in the pure glucose solution in presence of the Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 6−12, reaction temperature 60 °C.

Lv et al. (2015), introduced in section 2.2.1, investigated glucose isomerization into fructose in presence of SBA resin which led to fructose yield of 25 % after 12 hours when initial pH of glucose solution was 8. The pure glucose solution reached almost the same fructose yield of 24 % after 2 hours in presence of the Na/Ca/Mg-loaded WAC resin under the same reaction temperature, but initial pH 12.

Also, pH increased more in presence of the Na-loaded resin than in presence of the Na/Ca/Mg-loaded resin during experiment which could explain higher fructose fractions with the Na-loaded resin. Measured pH values after experiments are listed in Table XI. The Na-loaded resin increased pH of glucose solution more. For example, in presence of the Na-loaded resin, pH of glucose solution increased to 9−11 after 20 minutes and 2 hours in samples which initial pH was 6,6−9,2 while at the same conditions pH was measured only to around 8−9 in presence of the Na/Ca/Mg-loaded resin. pH of the solution decreased close to neutral after longer reaction time as 6 and 24 hours with both Na- and Na/Ca/Mg-loaded resins.

Table XI Measured pH values of the pure glucose solution before and after addition of the Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, reaction temperature 60 °C.

pH before resin addition	6	8,3	8,9	9,9	11	12
pH (20 min)	8,7	8,7	8,5	8,5	10,1	11,3
pH (2 h)	8	8,1	8,2	8,4	9,6	10,7
pH (6 h)	7,9	7,9	7,9	8	9	9,7
pH (24 h)	7,7	7,7	7,6	7,7	8,1	8,2

As mentioned earlier, pH of the pure glucose solution might be increasing in presence of the Na-loaded resin because position of equilibrium, in equation (5), lies on the right hand side when NaOH is produced. Most likely, position of equilibrium lies on the left hand side when the resin is in a divalent ion form. Only 35 % of the Na/Ca/Mg-loaded resin was in monovalent form, i.e. Na-loaded, which could explain why pH did not increase as much as with completely Na-loaded resin. Figure 39 shows total concentration of glucose and fructose as a function of time with varying initial pH values of the pure glucose solution. Similar level of decreasing in total concentration of glucose and fructose is observed in presence of both Na- as well as Na/Ca/Mg-loaded resins.

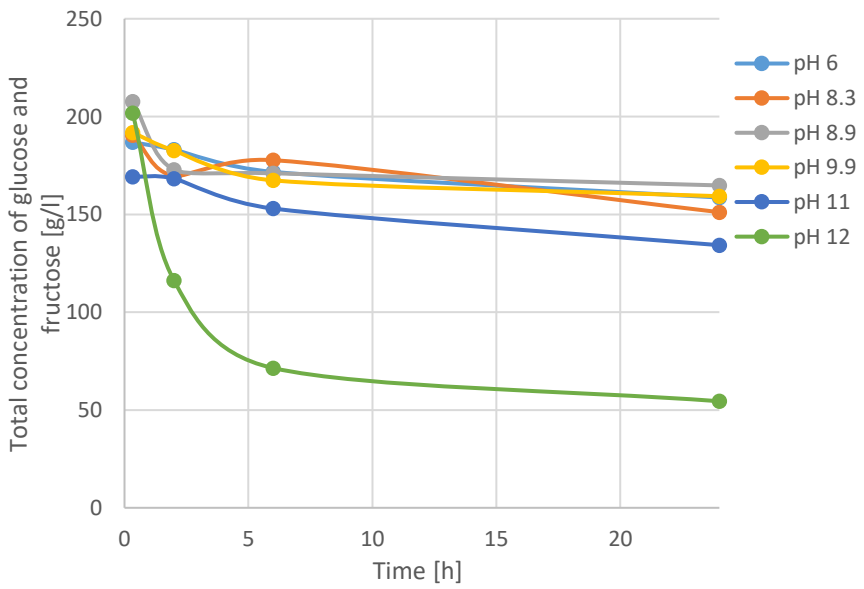

Figure 39 Effect of reaction time and pH on degradation of glucose and fructose in the pure glucose solution in presence of the Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 6−12, reaction temperature 60 °C.

It can be seen from Figure 37 that concentration of carboxylic acids is about at the same level with both resins in samples where initial pH of glucose solution was between pH 6−10. However, slightly higher carboxylic acid concentration was detected in a sample which initial pH was 12 and carried out in presence of the Na-loaded resin (6,7 g/l) compared to the Na/Ca/Mg-loaded resin (5,5 g/l). More than a half of total amount of carboxylic acids was identified to consist of acetic acid.

Figure 28c showed 7 reaction tubes filled with glucose solution and the Na/Ca/Mg-loaded WAC resin where initial pH was varying from 6,6 to 12 after 6 hours. When comparing Figure 28b and Figure 28c, it can be seen that the color of glucose solutions was darker in presence of the Na-loaded resin than in presence of the Na/Ca/Mg-loaded resin, especially the solution is more yellow in reaction tubes where pH of glucose solution was adjusted to vary between 6−10 at the beginning. This may be due to the fact that in presence of the Na-loaded resin, pH of glucose solutions increased more in samples which initial pH was 6−10 than with the Na/Ca/Mg-loaded resin, as discussed earlier. Based on literature, more alkaline conditions should promote glucose degradation into acidic products and darkening of a sugar solution.

Glucose solution 1 and 2 with the Na/Ca/Mg-loaded WAC resin

Experiments with GS 1 and GS 2 and the Na/Ca/Mg-loaded WAC resin was carried out in the same way as earlier with the pure glucose solution. Outcome of both experiments, GS 1 and GS 2 carried out in presence of the Na/Ca/Mg-loaded resin, are very similar as experiment with the pure glucose solution and therefore diagrams of fructose fraction, fructose yield and total concentration of glucose and fructose as a function of time and table of pH values after experiment are presented in Appendix II.

Purity of glucose solution do not have an effect on glucose isomerization or degradation. It can be concluded that these two WAC resins do promote glucose isomerization into fructose but at the same time creates unstable environment for these monosaccharides which leads to low fructose yields.

4.2.4 Catalytic effect of metal addition on isomerization of glucose

Besides pH, reaction time and temperature, metal addition might have promoting effect on glucose isomerization into fructose. Based on literature, addition of some metals as aluminate and borate have led to improved fructose yields in alkaline conditions. Effect of three different metals; calcium, magnesium and sodium, on glucose isomerization and degradation was investigated. Earlier, in section 4.2.2, studied WAC resin was loaded with these same metals. Glucose content of the pure glucose solution turned out to be between approximately 280−320 g/l in these experiments. Reaction was allowed to carry on for 20 min− 24 h at 60 °C and initial pH of the pure glucose solution was adjusted to vary between 6−12.

Figure 40 shows fructose fraction and yield as a function of time when metal chloride content of the pure glucose solution was 0,2 mol/l. Profiles of fructose fraction in experiments where $CaCl_2$ or NaCl was added to the pure glucose solution were quite similar as in the experiments without the metal addition. Mendicino (1960), Barker et al. (1975) and Bruijn et al. (1987), introduced inn section 2.2.1, have done experiments where the yield of fructose was significantly increased by adding metals as borates or aluminates in alkaline glucose solution. Barker et al. (1975) succeed to increase fructose yield to even 56 % with borate addition and Despax et al. (2013) to 41 % with aluminate addition. These improved yields of fructose are known to be result of fructose forming a complex with borate or aluminate which leads to glucose–fructose reaction equilibria to shift towards fructose. It can be speculated that Ca^{2+} and Na^{+} ions were not able to form complexes with fructose and shift the reaction equilibria which can be due to these metals being differently charged. Borate and aluminate are anions while metal ions used in these experiments are cations.

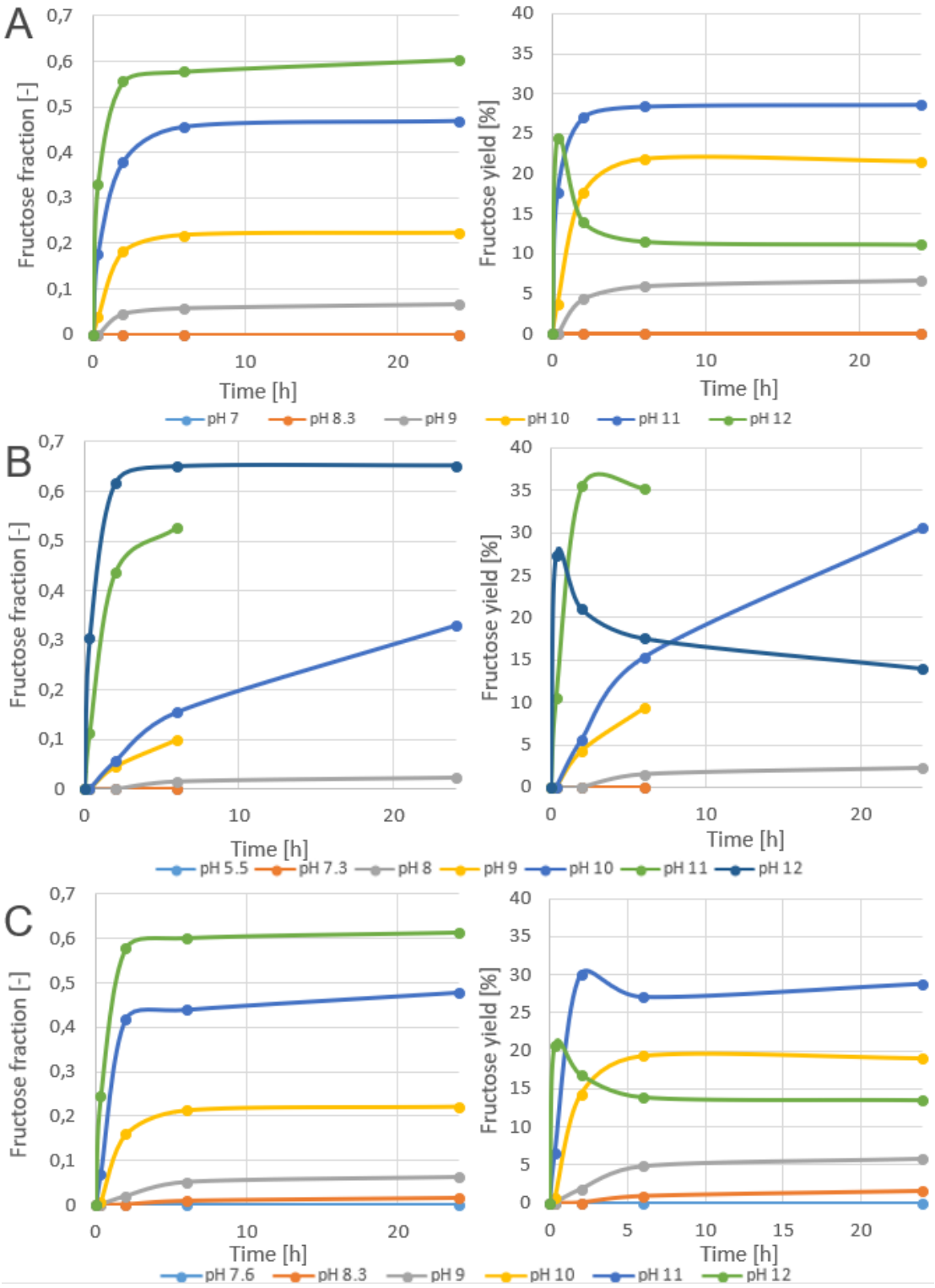

Figure 40 Fructose fraction (left) and yield (right) in the pure glucose solution in presence of 0,2 mol/l A) $CaCl_2$, B) $MgCl_2$ or C) NaCl. Reaction time 20 min−24 h, initial glucose concentration 280−320 g/l, initial pH 6−12, reaction temperature 60 °C.

However, addition of $MgCl_2 \cdot 6H_2O$ into the pure glucose solution led to higher yields of fructose than $CaCl_2$ or NaCl addition. For example, the highest fructose yield of 35 % with

$MgCl_2$ addition was detected in a sample which initial pH was 11 and reaction time was 2 or 6 hours. While the highest fructose yield of 29 % and 30 % was observed with $CaCl_2$ or NaCl addition, respectively.

Measured pH values after 20 min, 2 h, 6 h and 24 h are listed in Table XII. In experiments where $CaCl_2$ or NaCl was added, pH decreased during experiment as earlier in the experiment with the pure glucose solution without a metal addition; pH decreased to neutral due the formation of acidic compounds. In the experiment where $CaCl_2$ was added, pH decreased even to 4−5 after 24 hours which could indicate that more acidic compounds were formed than in the experiments without metal addition. However, in the experiment where magnesium was added, pH decreases slower over time and did not decrease that low. For example, in samples which initial pH was adjusted to 10 and 12, pH decreased only to 9 and 10 after 24 hours. In the same conditions with calcium addition, pH decreased to 6 and 4. Higher pH values in the magnesium containing samples could explain higher fructose yields.

Table XII Measured pH values of glucose solution after experiment. Effect of metal addition on glucose isomerization was investigated with addition of 0,2 mol/l of $CaCl_2$, $MgCl_2$ and NaCl.

$CaCl_2$

Initial pH	**7**	**8,3**	**9**	**10**	**11**	**12**
pH (20 min)	7,7	8,1	8,8	9,6	10,4	11,5
pH (2 h)	7,8	7,4	8,3	8,9	9,7	10,3
pH (6 h)	4,8	5,5	5,8	6,6	7,6	8,7
pH (24 h)	5,7	5	4,5	6	6,6	4,1

$MgCl_2$

Initial pH	**5,5**	**7,3**	**8**	**9**	**10**	**11**	**12**
pH (20 min)	4,8	6,4	7,9	8,9	9,6	10,9	11,9
pH (2 h)	7,5	7,2	7,8	8,8	9,5	10,2	10,8
pH (6 h)	5,2	6	6,5	7,7	9,4	9,9	10,1
pH (24 h)			7,1		9		9,9

NaCl

Initial pH	**7,6**	**8,3**	**9**	**10**	**11**	**12**
pH (20 min)	6,7	8,2	8,8	9,9	10,9	11,9
pH (2 h)	7	8	8,7	9,6	10,2	10,8
pH (6 h)	6,1	7	7,9	7	7,8	8,5
pH (24 h)	6,4	5,9	5,7	5,7	6,6	7,6

Figure 41 shows total concentration of glucose and fructose as a function of time. The total concentration decreased with and without metal addition similarly and about at the same

level. Total concentration decreased remarkably only in samples which initial pH was 11 and 12.

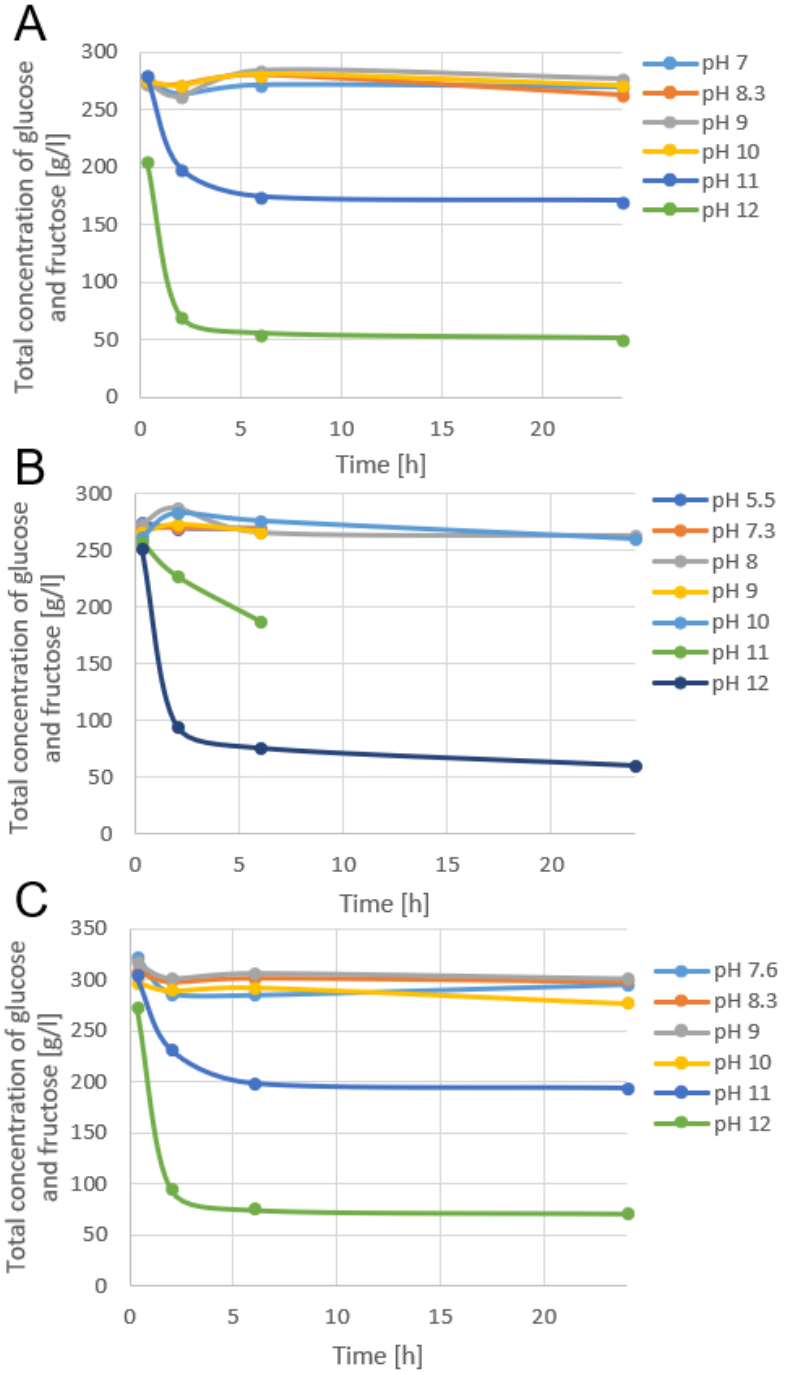

Figure 41 Effect of reaction time and pH on degradation of glucose and fructose in the pure glucose solution in presence of 0,2 mol/l A) $CaCl_2$, B) $MgCl_2$ or C) NaCl. Reaction time 20 min−24 h, initial glucose concentration 280−320 g/l, initial pH 6−12, reaction temperature 60 °C.

In experiments by Bruijn et al. (1987), discussed in section 2.2.1, the degradation rate of glucose into acidic compounds was approximately five times higher after calcium addition than the increase of its isomerization reaction rate. This phenomenon is not observed when

comparing the outcome between experiments where calcium chloride was and was not added to the pure glucose solution. However, glucose-calcium molar ratio was higher in the experiment carried out by Bruijn et al. (1987) which has probably had an effect on the increased degradation rate of glucose.

Concentration of calcium, magnesium and sodium was analyzed from samples which reaction time was two hours by microwave assisted digestion followed by ICP. Overall, metal content stayed constant regardless of initial pH. Calcium concentration stayed about 7 g/l and magnesium 5 g/l which is the same concentration as at the beginning of experiments. Sodium concentration stayed also about at the same level (about 5 g/l) in samples which initial concentration was between 7,6 and 9 but then, of course, began to increase due to NaOH addition.

Carboxylic acid content was analyzed from all the samples which reaction time was 6 hours. Concentration of carboxylic acids is presented in Figure 42 as a function of initial pH. Concentration profiles of carboxylic acids in samples where calcium and sodium were added is somewhat similar as in the previous experiment without metal addition. Carboxylic acid content starts to increase in samples which initial pH is 10 or higher. However, carboxylic acids were hardly detected in samples where magnesium chloride was added, the highest carboxylic acid content was only 0,3 g/l.

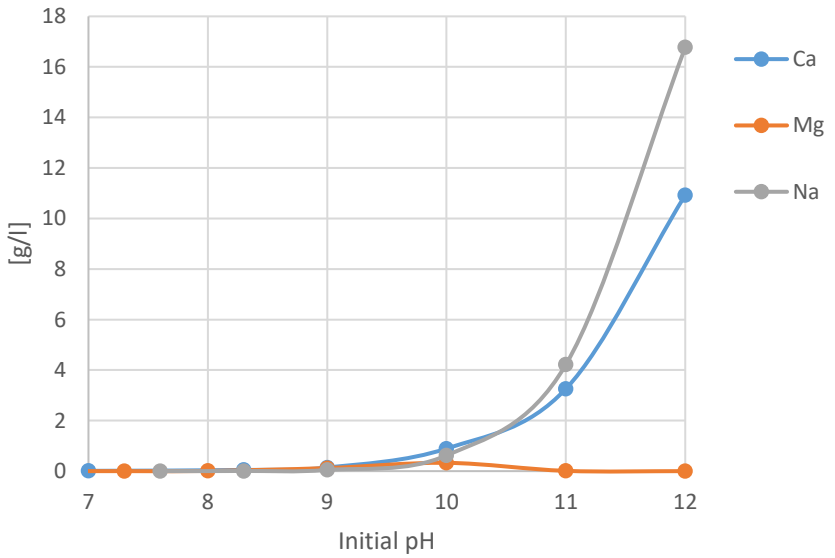

Figure 42 Effect of initial pH on formation of organic acids in the pure glucose solution in presence of Ca, Mg or Na ions. $CaCl_2$, $MgCl_2$ or NaCl concentration 0,2 mol/l, initial glucose concentration 280−320 g/l, initial pH 6−12, reaction time 6 h, temperature 60 °C.

At the beginning, calcium precipitated in the pure glucose solution which initial pH adjusted to 10−12, but after the experiment no precipitation was observed in any sample. Precipitation was observed before and after experiments only in magnesium-containing samples. The precipitate also changed in color from white to orange/dark during the experiments. It can be speculated that at the beginning magnesium chloride was precipitated but during experiments dissolved to water due to acidic compound formation and finally precipitated back with the acidic compounds. This could be one possible explanation why almost any carboxylic acid compounds were detected. Lower amounts of carboxylic acids could be the reason why pH did decrease less than in samples where calcium or sodium was added.

It can be concluded that at least addition of calcium chloride and sodium chloride did not affect significantly on glucose isomerization into fructose or degradation into acidic compounds. However, addition of magnesium chloride increased fructose fraction which could be due to magnesium ion forming a complex with fructose and thus shifting the reaction equilibrium towards fructose formation. More likely, higher fractions of fructose could be a result of more alkaline conditions since pH did not decrease during experiments as in the case of calcium and sodium chloride addition. This could be due to magnesium being precipitated with the formed acidic compounds during experiment. Although, magnesium addition did lead to improved fructose fractions, it cannot be considered as an effective catalyst for producing fructose since decrease of total concentration of glucose and fructose decreased from about 280 g/l to 60 g/l at lowest and gained fructose yield was low.

4.2.5 Effect of pH adjustment over time on fructose formation

Based on results from all the previous experiments, pH has a significant effect on glucose isomerization and degradation. In general, fructose fraction has been higher in samples which initial pH is more alkaline. However, pH is commonly decreasing during experiments due to glucose and/or fructose degradation into acidic compounds. Therefore, total concentration of glucose and fructose also decreases. However, usually a little or no changes on gained fructose yield or total concentration have been observed after 6 hours. The aim of this following experiment is to investigate effect of pH adjustment during experiments on glucose isomerization and degradation.

The pure glucose solution and GS 4 was used in these experiments. Reaction temperature of 50 °C was chosen to this experiment based on the results gained from earlier experiments in section 4.4 where effect of temperature on glucose isomerization and degradation was investigated. Glucose did not isomerize into fructose at 40 °C as efficiently as at 60 °C. However, glucose and/or fructose degraded into acidic and unknown compounds more at 60 °C. Also, pH was chosen based on earlier results. As concluded earlier, pH 10 and 11 might be optimal conditions to investigate glucose isomerization into fructose. Therefore, at the beginning of experiment pH of the pure glucose solution was adjusted to 10,5 and original pH of GS 4 was 11 which was not adjusted from that. pH was adjusted back to 10,5 every three hours in both glucose solutions but the last samples which reaction time was 24 hours was allowed to react for the last 12 hours without pH adjustment. Figure 43 shows fructose fraction and yield as a function of time.

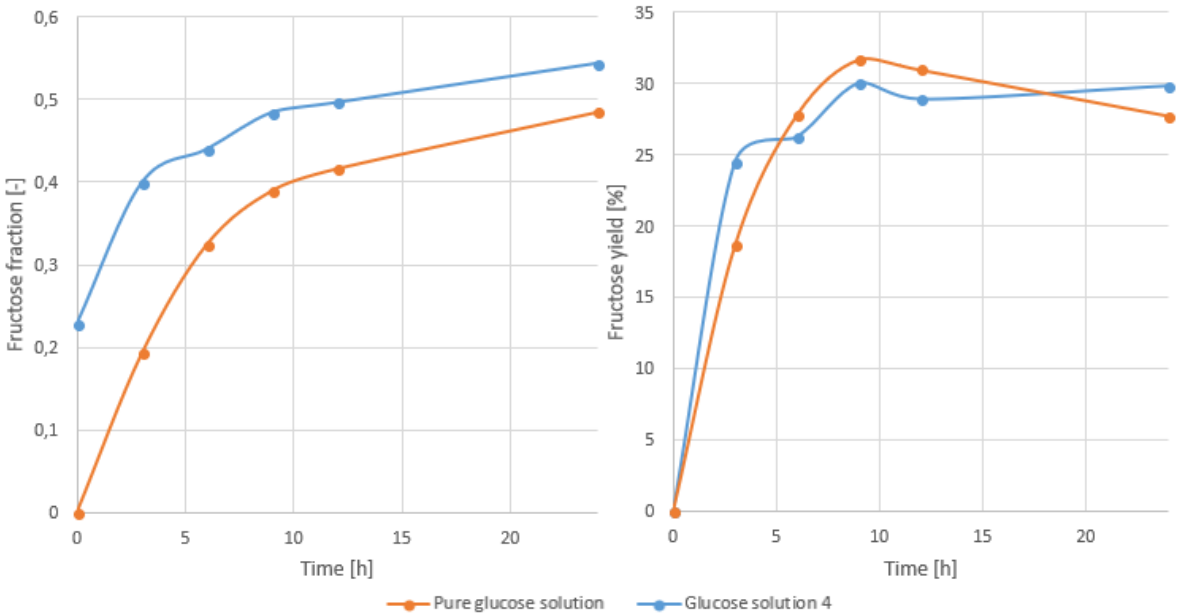

Figure 43 Fructose fraction (left) and yield (right) in the pure glucose solution (250 $g_{glucose}$/l) and GS 4 (142 $g_{glucose\ and\ fructose}$/l). pH was adjusted to 10,5 every three hours and reaction temperature was 50 °C.

No experiments have been carried out before in this experimental part at 50 °C or when solution pH was 10,5, so it is not possible to compare accurately how the pH adjustment during the experiments has affected. However, unlike in any other previous experiment, in this case the reaction time had more effect on a formation of fructose due the pH never neutralized. Most of the time pH was over 10 which is observed as a great reaction condition for glucose isomerization. The highest fructose yield of 32 % and 30 % was detected in the

pure glucose solution and in GS 4, respectively. Fructose yield began to decrease slightly after 9 hours when it had reached its maximum.

Unlike fructose yield, fructose fraction increased constantly over time. The highest fructose fraction for the pure glucose solution of 0,49 was detected in a sample which initial pH was 12 after 24 hours and fructose yield was 27 % in that trial point. That is relatively high fructose fraction when compared to the highest fructose fraction for the pure glucose solution carried out at 60 °C in section 4.2.1, which led to fructose fraction of 0,44 in initial pH 11 after 24 hours. Fructose yield was about in the same level with and without pH adjustment. The pure glucose solution which pH was adjusted over time compared with the experiment in section 4.2.1 without pH adjustment resulted to slightly higher fructose fractions and the same level of fructose yield although temperature was 10 °C lower which demonstrates the importance of pH for isomerization reaction.

Figure 44 present total concentration of glucose and fructose as a function of time. Total concentration seems to decrease more slowly over time when pH was adjusted to 10,5 for every three hours during experiment under 50 °C compared with experiment carried out under 60 °C when initial was pH 11 without adjustment over time. However total concentration in these both experiments eventually decreases at the same level after 24 hours. For example, after 6 hours of reaction, total concentration of the pure glucose solution was 257 g/l in a sample which concentration was adjusted over time to 10,5 and carried out under 50 °C, but after 24 hours only 172 g/l was detected. While total concentration of 183 g/l was detected after 6 hours in the experiment where the pure glucose solution was carried out under 60 °C which initial pH was 11 and after 24 hours concentration had not decreased from that.

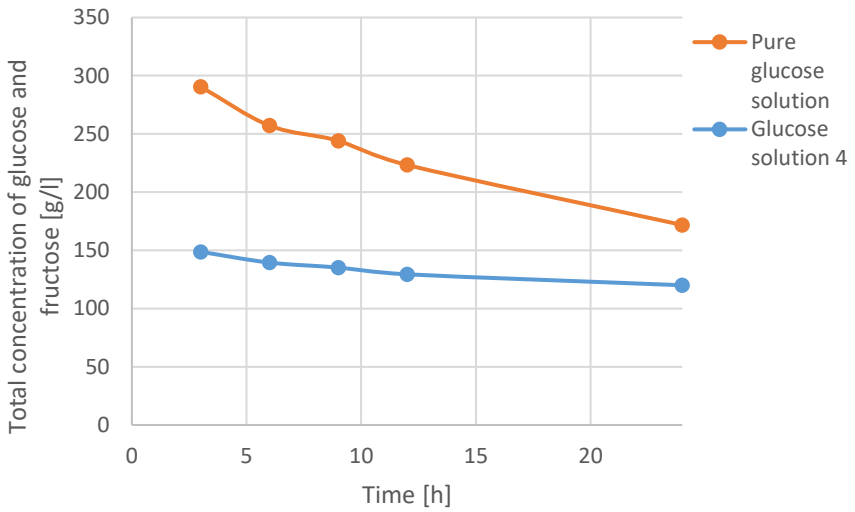

Figure 44 Effect of pH adjustment over time to 10,5 on degradation of glucose and fructose in the pure glucose solution (300 g/l) and GS 4 (142 $g_{glucose\ and\ fructose}$/l). Reaction time 3−24 h and temperature 50 °C.

GS 4 seem to be more stable against degradation than the pure glucose solution since total concentration decreased less in GS 4 during the experiment. Total concentration of GS 4 decreased only about 30 g/l in total during experiment while concentration of the pure glucose solution decreased even about 130 g/l in total. However, gained fructose yields were about at the same level between the pure glucose solution and GS 4. Glucose might degrade more actively into acidic compounds when glucose content of solution is higher.

5 FINAL CONCLUSIONS

It is important to understand effect of varying process conditions on reactions of glucose when developing an effective chemo-catalytic system for glucose isomerization or new promising applications utilizing glucose or its derivatives. Literature part of this thesis included review of glucose conversion into fructose introducing several chemical catalysts. In addition, the industrial process of HFCS was introduced including enzymatic conversion of glucose into fructose and chromatographic separation of fructose from glucose with SAC resin. Based on literature findings, the most important process parameters affecting glucose isomerization into fructose or degradation into acidic compounds are pH, reaction time and temperature. Therefore, effect of these process parameters was chosen to investigate on glucose isomerization and degradation in the experimental part of this thesis. In addition, some experiments were carried out with a metal addition and in presence of WAC resins.

Effect of pH on glucose isomerization was investigated within pH range of 6−12. Alkaline conditions are known to promote glucose isomerization into fructose and in most experiments only initial pH between 10−12 led to fructose formation. Initial pH 11 and 12 gave the highest fructose fractions which was commonly between 0,35−0,65. Initial pH 10 gave fructose fractions which was approximately between 0−0,2. In addition, pH was observed to change during the experiments. In most cases, pH decreased during reaction time which could be due to the formation of acid compounds. Generally, fructose formation slowed significantly or stopped after 6 hours when pH had decreased close to neutral or even below that. This indicates clearly the importance of solution pH on glucose isomerization.

Total concentration of glucose and fructose decreased during experiments which could refer to glucose and/or fructose degradation into acidic compounds since pH decreased also during experiments. The total concentration decreased significantly, even 200 g/l during experiments, when initial pH was 11 and 12. Carboxylic acids was commonly detected from samples which initial pH was 10−12. Based on literature findings, alkaline conditions catalyze also glucose and/or fructose degradation into acidic products besides isomerization reaction. In addition, fructose is known to be even more unstable under hot alkaline conditions than glucose.

Temperature was noted to play an important role on glucose isomerization into fructose and degradation into acidic compounds. Higher reaction temperatures clearly boost glucose isomerization. For example, the pure glucose solution which initial pH was 11 led to fructose fraction of 0,1 under 23 °C, 0,33 under 40 °C and 0,48 under 60 °C after 6 hours. Interestingly, pH hardly decreased in samples which were performed at 23 °C or 40 °C. However, in the experiments which were performed at 60 °C, pH of the pure glucose solution decreased three units from initial pH 10 and 11 after 6 hours. Logically, total concentration decreased less at 23 °C and 40 °C when pH did not decrease than at 60 °C when pH did decrease. This also reflects that decreasing total concentration is due to formation of acidic compounds and glucose and fructose are more unstable under higher reaction temperature. In addition, glucose isomerized and degraded faster under higher reaction temperatures.

Experiments where metals were added in to the glucose solution showed that at least addition of calcium and sodium ions does not affect significantly glucose isomerization or degradation. Addition of borates and aluminates has been proven to lead to higher yields of

fructose which is due to fructose forming a complex with metal ion which causes to glucose-fructose reaction equilibria to shift towards fructose. Apparently, fructose was not able to form a complex at least with sodium or calcium ion which might be due to these metals being differently charged than borates and aluminates or due to low metal content. Metal content of glucose solution was only 0,2 mol/l. Therefore, a topic for further investigations could be to perform similar experiments with higher metal content to see whether it affects the formation of complexes between fructose and metal ions. However, addition of magnesium increased fructose fraction which could be due to magnesium ion forming a complex with fructose. More likely, higher fructose fractions was a result of more alkaline conditions since pH did not decrease during experiments as in the case of calcium and sodium addition. This could be due to magnesium being precipitated with the formed acidic compounds during experiment. Although, magnesium addition led to improved fructose fractions, it cannot be considered as an effective catalyst for producing fructose since decrease of total concentration of glucose led to low fructose yields as before.

In addition, glucose isomerization was studied in presence of Na- and Na/Ca/Mg-loaded WAC exchange resins. Higher fructose fractions were gained in presence of the Na-loaded resin compared to in presence of the Na/Ca/Mg-loaded resin or in the experiment without the resin. Again, the link between pH of the glucose solution and fructose formation could explain this. The Na-loaded resin was probably more selective than the Na/Ca/Mg-loaded resin for producing hydroxide in the reaction with water which resulted more alkaline conditions and thus promoted glucose isomerization. However, the yield of fructose was low since alkaline conditions catalyze also the formation of acidic compounds.

Since pH of the glucose solution turned out to be a significant factor affecting to reactions of glucose, experiments where pH was adjusted over time was chosen to perform. Experiments were carried out at 50 °C and pH of the glucose solution was adjusted to 10,5 every three hours. No experiments have been carried out before under these exact same conditions without pH adjustment over time, so it was not possible to compare accurately how the pH adjustment affected. Therefore, experiments carried out under the same reaction conditions without the pH adjustment would be reasonable for further investigations. However, it seemed that pH adjustment during experiment did lead to slightly improved fructose fractions but at the same time total concentration of glucose and fructose also decreased more so that the fructose yield was not improved. However, total concentration of

glucose and fructose decreased only approximately 30 g/l in GS 4 during experiment while total concentration in the pure glucose solution decreased even about 130 g/l.

Initial concentration of glucose solution might also affect to glucose and/or fructose degradation and glucose isomerization since initial glucose concentration of the pure glucose solution was about 300 g/l and 110 g/l in GS 4. Therefore, effect of varying glucose content on glucose isomerization and degradation would be reasonable for further investigation. It would be appropriate to study how temperature and alkalinity are affecting to glucose isomerization and degradation depending on glucose content and investigate reaction speed of isomerization and degradation with varying glucose content.

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APPENDICES

Appendix I	Composition of GS 1−4.
Appendix II	Fructose fraction, yield and total concentration of glucose and fructose as a function of time and concentration of carboxylic acids and furans as a function of time after 6 h in GS 1 and GS 2 with Na/Ca/Mg-loaded WAC exchanged resin.
Appendix III	Fructose fraction, yield and total concentration of glucose and fructose as a function of time in GS 3 and GS 4.

Table I Composition of GS 1 and GS 2.

	Unit	GS 1	GS 2
Lignin, soluble	mg/l	19582	16582
Glucose	mg/l	272107	285056
Fructose	mg/l	0	4779
Carbohydrates	mg/l	425655	448140
pH	-	7,4	5,3
Conductivity	mS/cm	5,9	6,4
Carboxylic acids	mg/l	17619	7871
Furans, total	mg/l	843	171

Table II Composition of GS 3 and GS 4.

	Unit	GS 3	GS 4
Lignin, soluble	mg/l	1909	1542
Calcium	mg/l	0,1	3
Magnesium	mg/l	0,2	4,5
Sodium	mg/l	482	4021
Glucose	mg/l	127197	109866
Fructose	mg/l	0	32469
Carbohydrates	mg/l	195099	167767
Conductivity	mS/cm	1,2	6,2
pH	-	7,4	10,9
Carboxylic acids	mg/l	1396	2349
Furans, total	mg/l	270	8

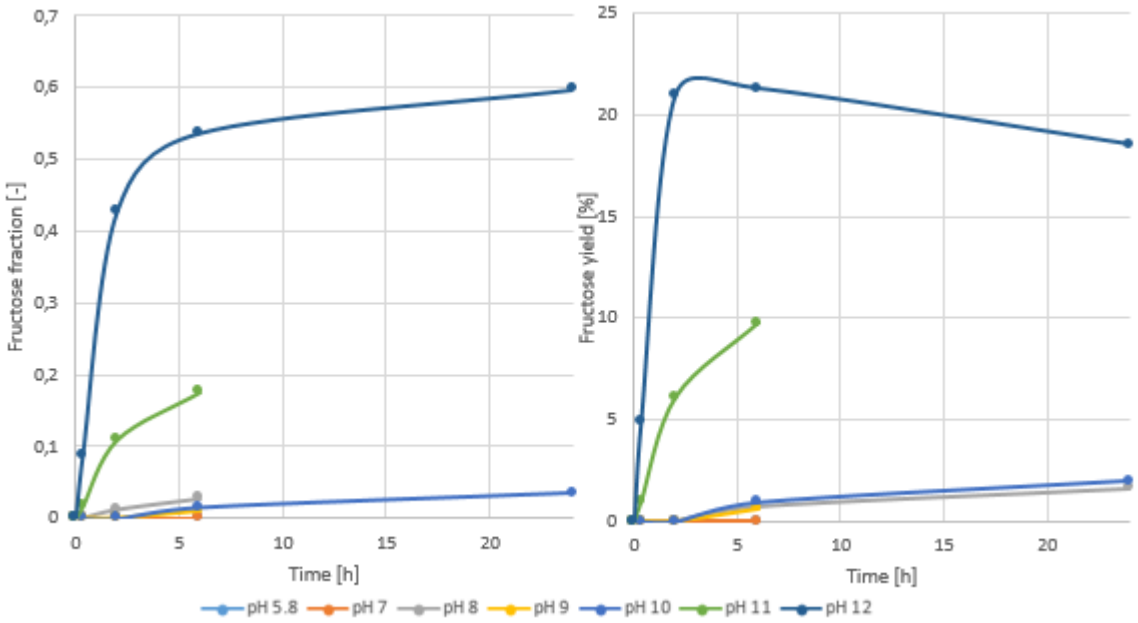

Figure 1 Fructose fraction (left) and yield (right) in GS 1 in presence of Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 5,8−12, reaction temperature 60 °C. Only solutions which initial pH was 8,2; 10,2 and 12 were allowed to react for 24 hours.

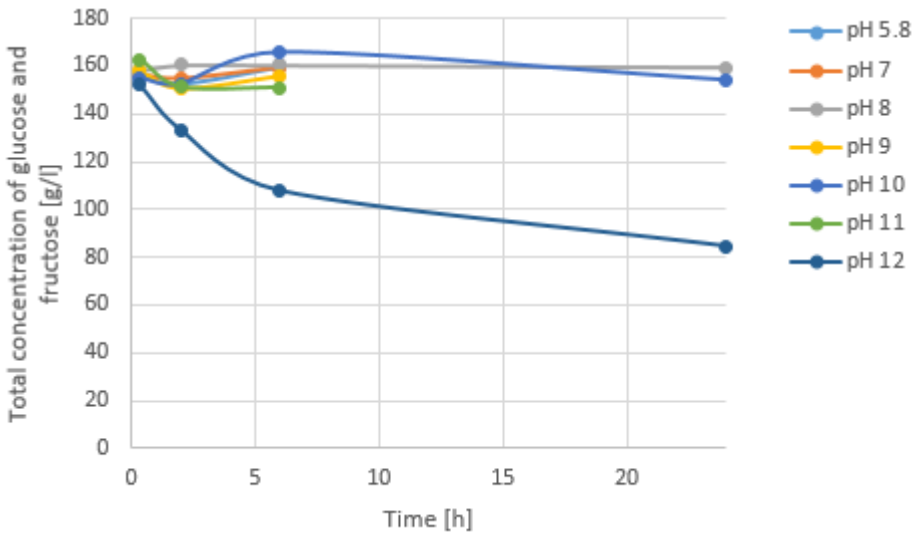

Figure 2 Effect of reaction time and pH on degradation of glucose and fructose in GS 1 in presence of Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 5,8−12, reaction temperature 60 °C. Only solutions which initial pH was 8,2; 10,2 and 12 were allowed to react for 24 hours.

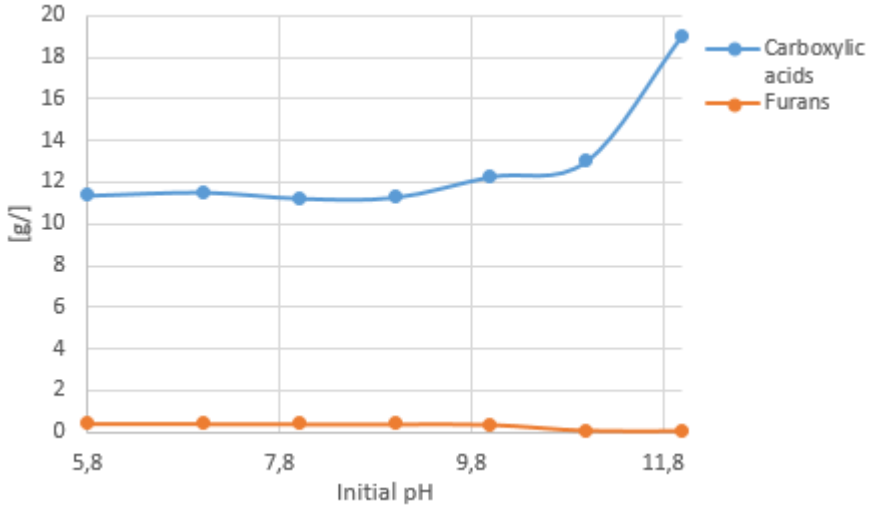

Figure 3 Effect of pH before resin addition on formation of organic acids in GS 1 in presence of Na/Ca/Mg-loaded WAC resin. Reaction time 6 h, pH before resin addition 5,8−12, temperature 60 °C.

Table III Measured pH values of GS 1 before and after addition of Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, reaction temperature 60 °C. Only solutions which initial pH was 8; 10 and 12 were allowed to react for 24 hours.

Initial pH	5,8	7	8	9	10	11	12
pH (20 min)	6,5	6,7	6,8	6,8	7,2	9,7	11
pH (2 h)	6,9	6,8	6,8	6,9	7,2	8,5	10,1
pH (6 h)	6,9	6,9	6,9	6,8	7	7,6	9,5
pH (24 h)			6,8		6,8		8

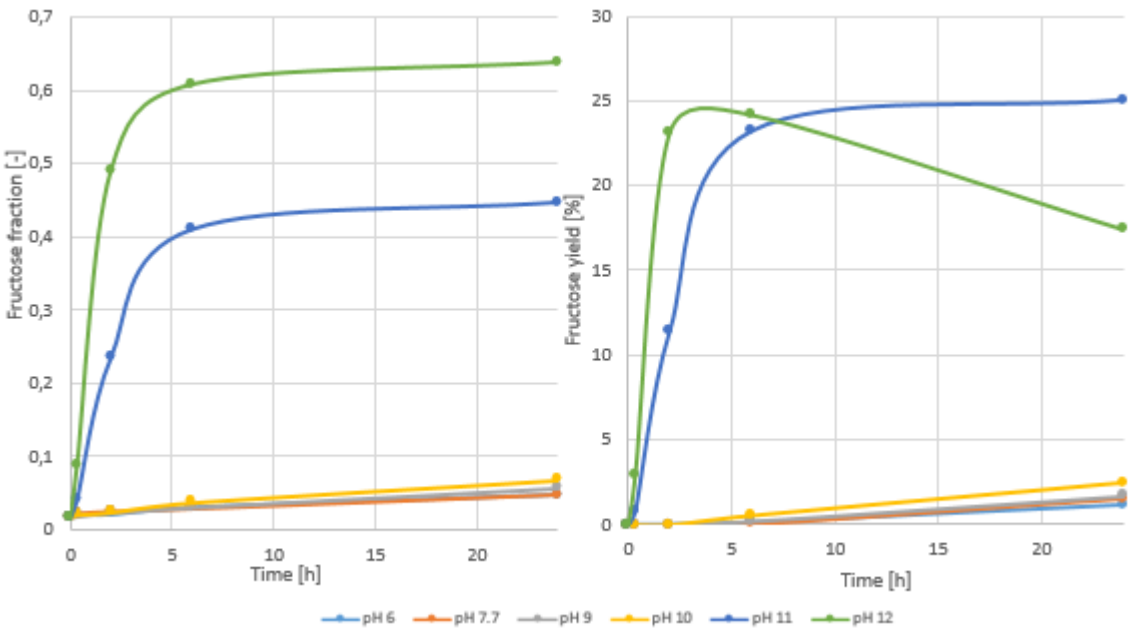

Figure 4 Fructose fraction (left) and yield (right) in GS 2 in presence of Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 6−12, reaction temperature 60 °C.

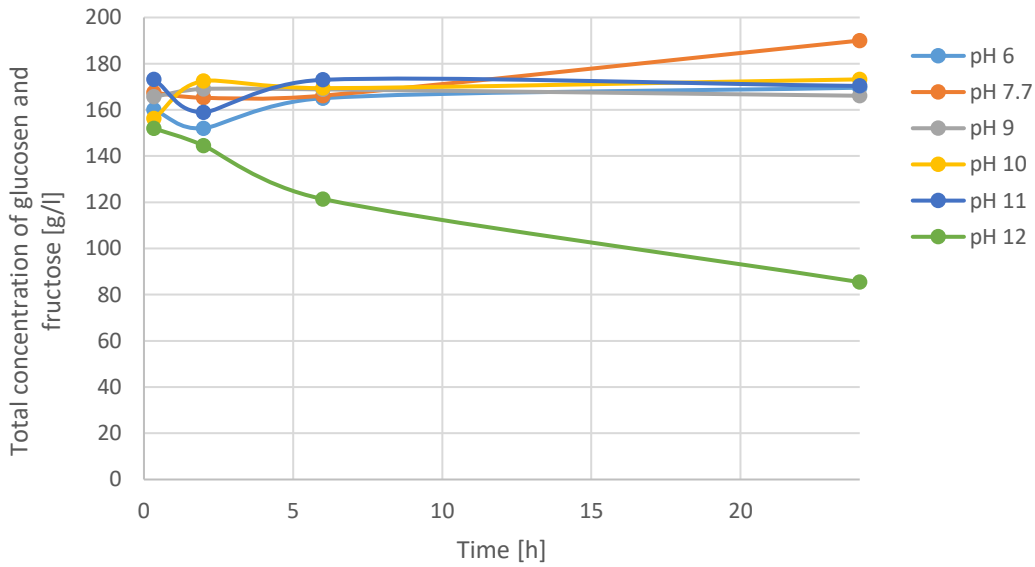

Figure 5 Effect of reaction time and pH on degradation of glucose and fructose in GS 2 in presence of Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, pH before resin addition 6−12, reaction temperature 60 °C.

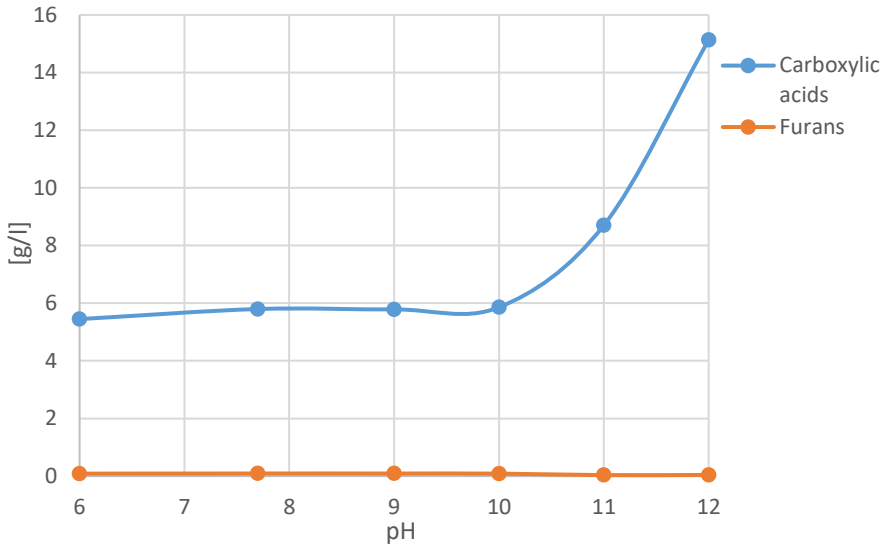

Figure 6 Effect of pH before resin addition on formation of organic acids in GS 2 in presence of Na/Ca/Mg-loaded WAC resin. Reaction time 6 h, pH before resin addition 6−12, temperature 60 °C.

Table IV Measured pH values of GS 2 before and after addition of Na/Ca/Mg-loaded WAC resin. Reaction time 20 min−24 h, reaction temperature 60 °C.

Initial pH	**6**	**7,7**	**9**	**10**	**11**	**12**
pH (20 min)	6,9	7,1	7,2	7,5	10,7	11,4
pH (2 h)	7	7	7,2	7,1	9,7	10,4
pH (6 h)	7	7,2	7,3	7,4	8,4	9,9
pH (24 h)	6,8	7	6,9	7,2	7,5	8,4

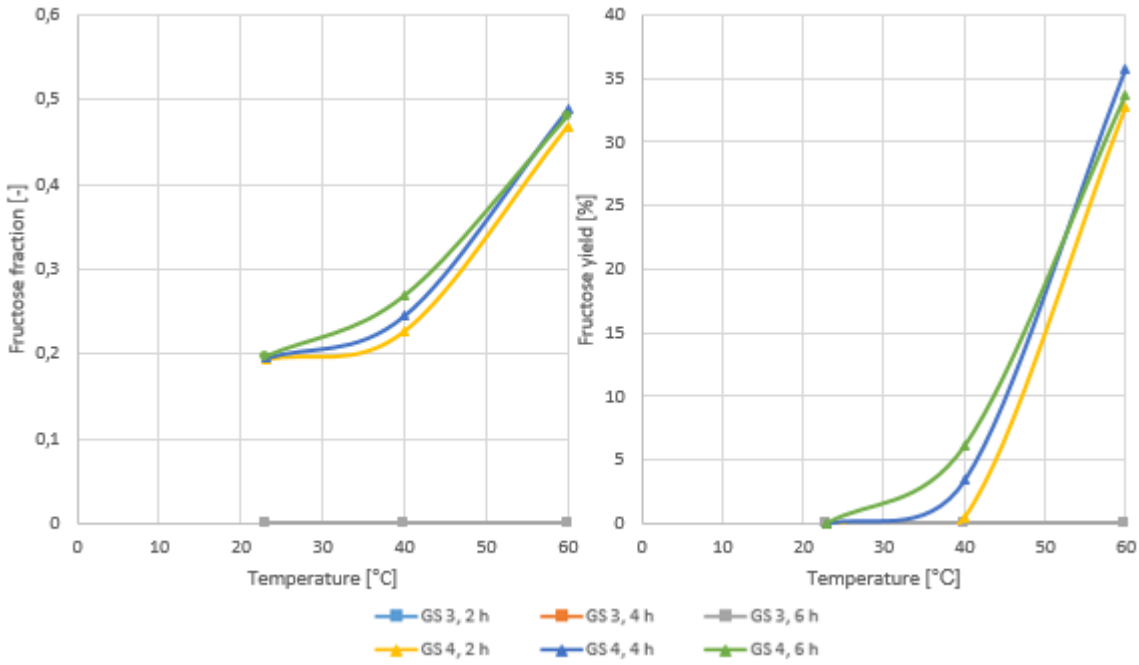

Figure 7 Fructose fraction (left) and yield (right) in GS 3 (▲) and GS 4 (■) when reaction temperature was 23, 40 and 60 °C. Reaction time 2−6 h, initial total concentration of glucose and fructose 127 g/l (GS 3) and 142 g/l (GS 4), initial pH 10 (GS 3) and 11 (GS 4).

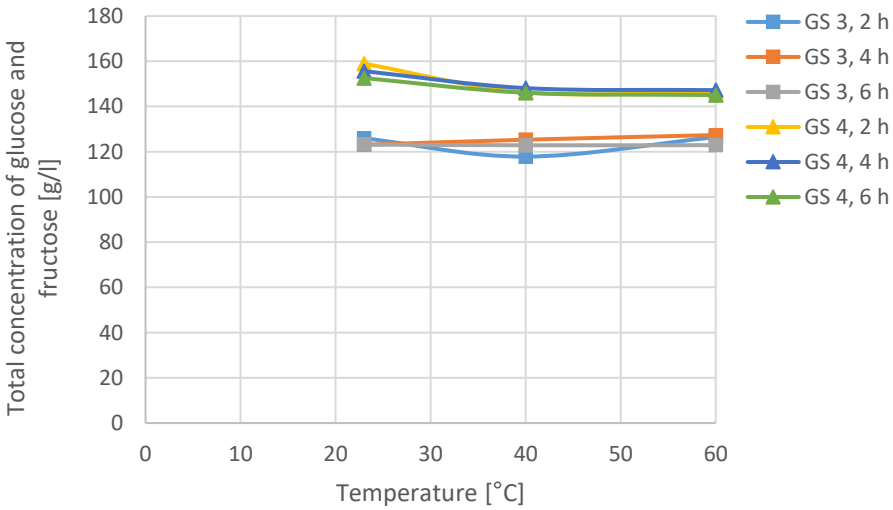

Figure 8 Effect of reaction temperature on degradation of glucose and fructose in GS 3 (▲) and GS 4 (■). Reaction time 2−6 h, initial total concentration of glucose and fructose 127 g/l (GS 3) and 142 g/l (GS 4), initial pH 10 (GS 3) and 11 (GS 4).

Table V Measured pH values of GS 3 and GS 4. Reaction time 2−6 h, initial total concentration of glucose and fructose 127 g/l (GS 3) and 142 g/l (GS 4), initial pH 8 (GS 3) and 11 (GS 4), reaction temperature 23, 40 and 60 °C.

Temperature [°C]	**23**	**23**	**40**	**40**	**60**	**60**
Initial pH	8	11	8	11	8	11
pH (2 h)	7,3	11,3	6,9	11,1	5,7	9,5
pH (4 h)	7	11,2	6,7	10,9	5,5	8,4
pH (6 h)	7	11,1	6,9	10,9	5,2	7,8