

Experimental Study of the Reaction Kinetics for the Production of Xylitol from Xylose Laden Waste Streams

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Declaration

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Master of Engineering at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

A handwritten signature in black ink, appearing to be 'K. S. S.', written over a horizontal line.

01 day of August 2024 at 16:07

Abstract

A statistically designed study was conducted to ascertain the kinetics of conversion of xylose to xylitol using borohydrogenation. Batch kinetic experiments were carried out for the reaction of xylose and sodium borohydride in the presence of sodium hydroxide solution. Analysis of xylose concentration-time data showed that the reaction order kinetics with respect to xylose follows a first-order rate law. This finding supports the mechanistic theory that the first hydride transfer is the rate-determining step. The study shows rate constants to be $3.44 \times 10^{-3} \text{ 1/s}$ and $9.76 \times 10^{-3} \text{ 1/s}$ at 25 °C and 60 °C. Additionally, the activation energy and frequency factor were 31.74 kJ/mol and 19146.72 kJ/mol, respectively. Furthermore, a two-level factorial design was used to determine the influence of specific parameters, namely: initial xylose concentration, temperature, and reaction time. The analysis of these reaction factors indicated that the most significant factor was time, followed by temperature and then concentration. Furthermore, interactions between time and concentration and the three-way interaction were significant.

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Chapter 1 : Introduction

1.1. Background

The investigation focuses on determining reaction kinetics to reduce xylose to produce xylitol, allowing optimal reactor design and deepening our understanding of the reaction mechanism. Sucrose ('table sugar') has been widely used as a sweetener for the past two centuries (Rodrigues *et al.*, 2023). However, sugar alternatives resulting from advances in food technology have changed the perception of sugar. Each alternative presents distinct functional characteristics that are both positive and negative for human health. Over the past century, xylitol has become popular due to its effect on carbohydrate metabolism and non-cariogenic qualities, allowing special dietary applications (Hyvönen, Koivistoinen and Voirol, 1982).

Figure 1-1 illustrates the three core structural components of plant biomass. Cellulose is a linear, crystalline polymer with repeating cellobiose units, providing strength and rigidity to the cell wall (Rodrigues *et al.*, 2023). Hemicellulose is a branched polymer composed of various sugars, contributing to cell wall flexibility and bonding. Lignin is an aromatic polymer made of monolignols, imparting rigidity and resistance to microbial degradation. Most chemical pulping processes follow the kraft technique to liberate cellulose required for paper production. This process produces waste streams termed 'black liquor'. A component of black liquor is hemicellulose which converts to various monosaccharides, including xylose, through hydrolysis. However, it is unexploited during downstream processing due to hemicellulose's low heating value (van Dam, Elbersen and Daza Montaña, 2018). This research aims to produce high-value products such as xylitol by reducing

xylose derived from black liquor. The reducing agent utilised in the reduction of xylose is sodium borohydride, which is a metal hydride. This method allows for a reduction reaction while minimizing the severe environmental and energy conditions associated with traditional xylitol production techniques. It achieves this by producing xylitol under milder conditions, requiring less energy, and eliminating the need for catalysts. Consequently, the study aligns with sustainable principles by retrofitting conventional pulping and xylitol production to become energy and resource-efficient.

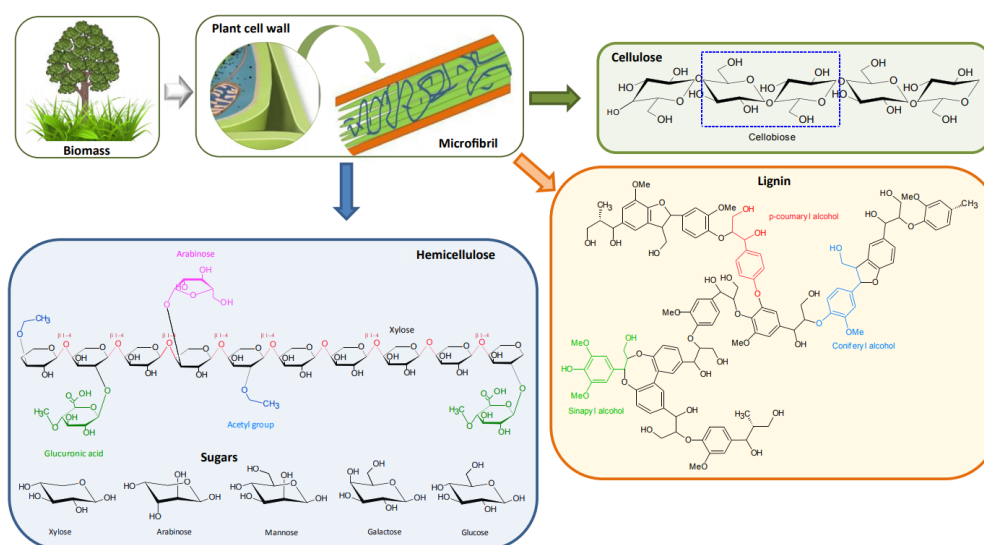


Figure 1-1: Overview of Hemicellulose Production

Kinetics are measured by initiating the reaction and monitoring the successive changes in xylose concentration as a function of time. The procedure follows a batch mixing experiment where a reaction is monitored by removing samples at set intervals. The reaction is terminated through quenching techniques, and samples are analysed using high-performance anion-exchange chromatography coupled with pulsed electrochemical detection (HPAEC-PED). This allows for both simple and effective quantification of alditols and monosaccharides in solution. Following

the same technique, a full factorial design is conducted to determine the effects of concentration, time and temperature, and the interactions between these parameters on xylose conversion.

1.2. Motivation

Currently, the rapidly growing human population have placed significant pressure on food resources and natural resources that are wasted due to inefficient industrial processes; hence, more emphasis has been placed on markets for bio-chemicals from non-food sources. Xylitol is consigned in this category as it is considered a second-generation sugar instead of first-generation sugars sourced from agricultural land. Thus focusing on these products will offer product value chains with a smaller carbon footprint allowing for greater environmental sustainability (SAPPI, 2015).

South Africa is among the countries with the highest obesity rates, with projections indicating a 47.7% increase in obesity among females and a 23.3% rise among males (van Vollenstee and van der Merwe, 2021). Data procured from the South African national surveys estimate that 68% of women and 31% of men are overweight or obese (Department of Health, 2024). Obesity-related diseases have been ranked in the top 5 risk factors for early death in South Africa, as 43% of deaths are caused by heart disease, certain cancers, strokes, and type 2 diabetes (Manafe, Chelule and Madiba, 2022). Furthermore, it is estimated that overweight and obesity account for 9% of total health expenditure in Africa and 13.2% globally. The direct costs are only a small part of the overall economic burden of overweight and obesity (Boachie *et al.*, 2022). It has been shown that each 500 ml fizzy drink has roughly ten spoons of sugar. As a result, the South African government plans on taxing drinks containing sugar according to the amount of sugar. This new tax

will lead to an approximate 20% tax on sugary drinks and is estimated to reduce obesity by 3.80% in men and 2.40% in women (Stacey, Tugendhaft and Hofman, 2017). This tax has led people to develop alternative sweeteners which are economically competitive. Xylitol has the advantage of providing a low glycaemic index because it is not actively transported through the intestinal tract and does not require insulin to be uptaken by the liver. The upper gastrointestinal tract slowly and incompletely absorbs xylitol, leading to a marginal effect on blood glucose (Rehman, Murtaza and Mushtaq, 2018). Besides, xylitol provides fewer calories than conventional sugars. A study conducted by Yamagata and coworkers on diabetic patients showed that an amount of 30-60 g/day resulted in no harmful effects on diabetic patients concerning fat and carbohydrate metabolism (Yamagata *et al.*, 1969). The control of blood glucose, lipid level and weight control are the main aims of managing diabetes. Therefore, xylitol is considered a suitable sweetener for diabetic patients (Pepper and Olinger, 1988).

As previously mentioned, there are several social, environmental and health benefits that xylitol can provide. The problem lies in current production techniques that are economically and environmentally taxing, leading to steep prices and small market volumes. As a consequence, only a small part of the human population has access to xylitol. This research involves studying the kinetics of this reduction reaction. Therefore, establishing a better understanding of the reaction mechanism allows for better design of suitable reactors and specifies optimal operating conditions, control methods, and auxiliary equipment. Existing literature only defines the reaction and gives scarce information on how it works, making this

study significant as it provides much-needed insight into a potentially low-cost production technique of xylitol.

1.3. Significance and scope

Alcohol preparation through the reduction of aldehydes and ketones is a prevalent reaction method utilised in organic synthesis. Despite its wide use, borohydride reductions have a complex nature, and the understanding of kinetic and mechanistic data is not adequately understood (Hudlicky, 1984).

Relatively few studies have examined the reaction between xylose using sodium borohydride. An early paper by Abdek-Akher, Hamilton and Smith provides evidence of its application and a patent by Nygren and coworkers established several methods for producing xylitol utilising sodium borohydride (Abdek-Akher, Hamilton and Smith, 1951; Nygren *et al.*, 1997). However, neither of these reports provides kinetic or optimisation data on the reduction of xylose. Therefore, this study closes this glaring knowledge gap by establishing kinetic data and investigated optimal xylose conversion over specified factors.

The study strictly focuses on the reduction of sodium borohydride and xylose in a sodium hydroxide solution. Other reducing techniques are not considered in this study. Other reducing agents include lithium aluminium hydride and Grignard reagents. The reason for the use of sodium borohydride is its practicality. This choice is further elaborated in the literature review.

This research provides a basis for future studies to reduce xylose using sodium borohydride; therefore, other known reduction techniques that potentially improve

xylose reduction are not included. These methods include the use of co-reagents, catalysts, microwave irradiation and electrolytic reduction.

The influence of specific parameters is studied, viz. initial xylose concentration and temperature at set reaction times. The estimation and the comparison of the parameter effects are accomplished by using a two-level factorial design. This experimental design is conducted to provide an empirical model for determining xylose conversion over these factors. Other factors were not studied, which include assessing the effects of non-protic and aprotic reaction solvents.

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Chapter 2 : Literature review

1.2.Introduction

There are two overall goals this study seeks to provide. Firstly, to provide information on the conflicting interpretation of mechanistic aspects of the reduction reaction between xylose and sodium borohydride and assess the application of this reduction technique in an industrial setting. Reduction reactions using sodium borohydride have been utilised for almost a century. However, there is scarce literature on reducing xylose in this manner, and the purpose of this investigation is to establish the reaction kinetics for this reaction. Furthermore, the study determines the conversion and yield over selected factors and compares these to other production techniques.

Several studies exist on borohydride reductions; however, the kinetic observations in studies have clouded the understanding of the mechanism, and there is little information on the performance of this xylitol-producing technique. Therefore, the following chapter will provide a background on sodium borohydride and xylitol as well as the scope of literature related to the investigation. Specifically, a focus on the mechanism, solvent effects and factors which affect reduction will be discussed. Finally, the setbacks and performance of other xylitol-producing techniques will be assessed.

2.1. Historical background

2.1.1. Sodium borohydride

Prior to discovering hydride reagents, reduction of carbonyl groups including aldehyde, ketones, and carboxylic acid esters was completed using chemical

mixtures to give corresponding carbinols. Specifically, mixtures of zinc dust with acetic acid, sodium amalgam with acetic acid and sodium in toluene were used (Brown and Krishnamurthy, 1979).

These methods had several drawbacks, including long reaction times, elevated temperatures, and low desired product yields. However, the discovery of metal hydrides and complex metal hydrides provided a much more feasible route for reducing aldehydes and other organic functional groups.

Diborane was discovered in 1912 by Stock, where production required preparation and hydrolysis of magnesium boride, followed by thermal decomposition of higher boron hydrides. This discovery led to studies that examined the reaction between diborane and typical carbonyl compounds (aldehydes, ketones, esters and acid chloride). Results revealed that diborane reacts rapidly with diborane at 0 °C. Furthermore, the dialkoxo derivatives produced are readily reduced by water to give the corresponding alcohols. Despite this being a considerable breakthrough in reduction, as it allowed for a satisfactory reduction at milder conditions, there were negligible reprint requests for the publications. The lack of interest was because diborane was a chemical rarity only available in small quantities (with a production rate of 0.5g/day). Additionally, diborane production required specialised equipment and complex preparative procedures. The bottleneck caused by diborane production led H.I. Schlesinger and Anton Burg to develop a more practical product synthesis.

At the same time, the National Defence Agency tasked Professor H.I. Schlesinger to develop new volatile compounds containing uranium. Before this, Schlesinger produced aluminium and beryllium borohydride, which led to the discovery of

uranium (IV) borohydrides that required diglyme to produce. However, diglyme was not feasible for mass production, and alternative reduction compounds were explored to develop uranium (IV) borohydride. Initially, lithium hydride was found to be a successful alternative. However, it was in short supply and required in the war effort, and the production of sodium borohydride became necessary. At this point, handling issues related to UF_6 were overcome, and there was no interest in uranium borohydride production. Although the Signal Corps required field generation of hydrogen and exploited sodium borohydride to achieve this. This demand for sodium borohydride urged further research into the compound, and the investigation for a proper separation solvent during production led to the discovery of its reduction properties. These events resulted in sodium borohydride being widely used as a hydrogenating agent to reduce organic compounds (Brown and Ramachandran, 1996).

2.1.2. Xylitol production

Professor Emil Herman Fischer and his assistant Rudolf Stahel were German chemists who extracted xylitol (reported as Xylit) from beech wood chips in September 1890 (Fischer and Stahel, 1890). Shortly after this, a French chemist named M.G. Bertrand isolated xylitol syrup by processing wheat and oat straw (Bertrand, 1891). Therefore, xylitol can be credited to these two groups of researchers. However, after its initial discovery, there was little interest in xylitol for the next half-century. During this period, xylitol was only utilised as a sweetener during World War II in Finland due to a sugar shortage. Engineers and researchers succeeded in developing a small-scale industrial process for the production of

xylitol. However, xylitol was no longer a necessity as peace times eased the sugar shortage.

During the 1970s, a few crucial studies facilitated the regenerating interest in developing xylitol commercially. The Turku Sugar studies involved 125 volunteers who replaced all sucrose in their diets. Two other test groups were included, one which ate fructose products strictly while the third group acted as the control group by remaining on a sucrose diet. This study showed that xylitol provides several health benefits, specifically oral health, over the other two sweeteners (Scheinin, Mäkinen and Ylitalo, 1976). This study drove further studies such as the Belize Trials in Central America (1989-1997), where foodstuffs were substituted with chewing gums. This study provided more substantial evidence of the benefits of xylitol over other used polyols, sorbitol, as a preventative agent for dental cavities. Specifically, children chewing xylitol gum led to 73% less tooth decay than those chewing sorbitol (Mäkinen *et al.*, 1995).

The first company to produce xylitol on a large scale was Finnish Sugar Ltd (now called Finland Gultor Ltd). This company arose from chromatographic separation technology development on an industrial scale to separate sucrose from molasses. This technology was then applied to the crystallisation technology of xylitol manufacturing. Xylitol markets grew in the late 1980s due to positive reports from dental literature, and by 1986, xylitol was approved as an additive for foods and declared safe for human use (Mäkinen, 2004).

Currently, xylitol is mainly utilised in confectionery, personal health products such as mouthwash and toothpaste, and the pharmaceutical industry as coating agents or

sweeteners in pharmaceutical products. Globally, more than 50 countries have approved xylitol in foods, pharmaceuticals, and oral health products. The production rate has increased more than 40 times since 1978 (Delgado *et al.*, 2018). In 2023, the global market for xylitol was estimated to be 713.4 million US dollars, with a projected annual growth rate of 4.3%. This projection would mean the market value would be over 1 billion US dollars by 2033, making xylitol a highly lucrative commercial investment (Future Market Insight Inc, 2023).

2.2. Properties of xylitol

Xylitol is a pentitol-type polyol composed of five hydroxyl groups, thus giving the compound a great affinity for water; compared to other polyalcohols, xylitol is the sweetest. Appendix B provides specific chemical and physical properties of xylitol. The standard molar enthalpy of formation of xylitol was derived as -1219.31 ± 0.3 kJ mol⁻¹. The melting temperature, standard molar enthalpy and entropy of this transition were determined to be 369.04 K, 33.26 ± 0.17 kJ mol⁻¹ and 90.12 ± 0.45 JK⁻¹.mol⁻¹, respectively. The stability of xylitol is not affected by pH; therefore, it is viable over a wide range of pH vital in our experimental work (Zhang, Wilson and Lee, 2016).

Xylitol exhibits a cooling effect which is caused by a negative heat of solution. Due to the additional energy taken from the solution, an endothermic reaction occurs to bring crystalline xylitol into the solution. This reaction provides pleasing organoleptic sensations when consuming xylitol. Compared to other sugar alcohols and glucose, xylitol provides the highest cooling effect. Polar groups of xylitol are highly solvated, similar to all sugars. The carbon-bonded hydrogen atoms (C-H) and oxygen atoms are firmly bound, and each OH-group is rapidly exchanged with

hydrogen atoms of water. Most sugars are known to transform water (especially with acids and alkalies). The carbon of the ketone or aldehyde group is generally responsible for these transformations. A sugar not made up of a reactive carbonyl group is considered more stable than those containing a reducing group. Thus, xylitol is suitable for food processes that require high temperatures. Furthermore, xylitol does not participate in Maillard reactions, allowing for particular applications in the food industry (Hyvönen, Koivistoinen and Voirol, 1982).

2.3. Features of sodium borohydride reductions

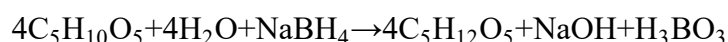
Sodium borohydride (also known as sodium tetrahydroborate/tetrahydridoborate) is an inorganic metal hydride with a white to greyish appearance in a crystalline powder form. This salt consists of the Na^+ cation and the BH_4^- anion. NaBH_4 has complex structural characteristics in that it is a Lewis acid and electrophilic at the metal centre. The B-H bond of NaBH_4 is polarised toward hydrogen. The hydrogen is, therefore, electron-rich and behaves like a hydride (H^-). Thermally sodium borohydride can be considered very stable as it slowly decomposes at temperatures upwards of 400°C (Stockmayer, Rice and Stephenson, 1955).

Furthermore, the BH_4^- ion has a tetrahedral structure with three stable polymorphs. NaBH_4 is a versatile reducing agent used in several industrial processes and is soluble in various protic and non-protic solvents and will be further discussed. Primary applications include organic and pharmaceutical synthesis, wastewater treatment, and paper pulp bleaching. Storing sodium borohydride in dry conditions is essential as it can rapidly absorb moisture from the air, leading to sodium metaborate and hydrogen formation. NaBH_4 can also store and generate hydrogen gas via a hydrolysis reaction (Yamamoto, 2003).

Some benefits of using sodium borohydride are that it is commercially available and is the least expensive metal hydride. It is a relatively safe compound regarding storage and usage as well as handling. Therefore, a plausible reducing agent in the production of xylitol.

As mentioned in chapter one, only two studies have looked at reducing xylose using sodium borohydride. An early paper by Abdek-Akher, Hamilton and Smith provides evidence of its application with a reported reaction time of 5 min and an acetylated product yield of 80 %. A patent by Nygren and coworkers established several methods for producing xylitol utilising sodium borohydride (Abdek-Akher, Hamilton and Smith, 1951; Nygren *et al.*, 1997). However, neither of these reports provide kinetic or optimisation data on the reduction of xylose.

The balanced equation below shows the theoretical stoichiometry of the overall reaction. As seen, the reagent xylose is 1:1 with the product xylitol. Thus, this should require 0.25 mol of sodium borohydride for every mol of xylose reacted.



In hydride reductions, it is customary to employ a slight excess of the reducing reagent if some of the metal hydride is unintentionally destroyed by reduction with hydroxyl-containing materials present in the reaction medium. Any excess sodium borohydride is destroyed by adding acid before the reaction workup and product isolation. The two quenching methods available are adding acid and rapidly reducing reaction temperatures, both of which are utilised in the following study.

Sodium borohydride permits for significant selectivity in reducing organic compounds owing to its mild reducing properties. It is generally accepted that the

order of reactivity of sodium borohydride among carbonyl groups is as follows ((Ward and Rhee, 1989; Brown and Ramachandran, 1996):

Table 2-1: Chemoselectivity of Sodium Borohydride

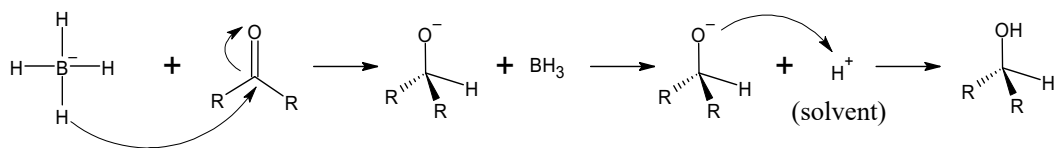
Reduction Difficulty	Functional Groups
Easily Reduced	Aldehydes>>Conjugated Enals>Ketones>Conjugated Enones>Acid Chlorides=Imines
Moderately Reduced	Esters, Epoxides, Lactones
Difficult to Reduce	Carboxylic Acids, Amides, Imides, Carbinol, Nitrile, Nitro Dehalogenation, Tosylehydrazone, Hydroboration and C-Calcogen Bond Cleavage

On the other hand, lithium aluminium hydride is a potent reducing agent that can reduce most organic functional groups. Sodium Borohydrides selectivity is an advantageous property as it allows for selective reduction of specific functional groups in the presence of others. Therefore, using sodium borohydride is more suitable in some instances compared to lithium aluminium hydride. These features are essential, especially when using biomass sources that can contain several organic functional groups.

2.4. Mechanism and kinetics

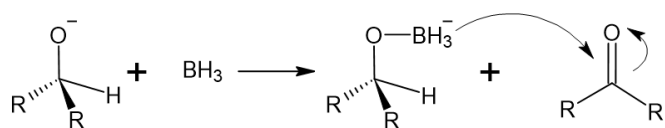
The reduction of carbonyl groups within aldehydes to corresponding alcohol has come to be ubiquitous in organic synthesis. Since its discovery, it is well recognised that carbonyl groups (including xylose) undergo a reduction reaction when in the presence of sodium borohydride. The following mechanism is the generalised idea for sodium borohydride-assisted reduction.

An aldehyde reaction with sodium borohydride consists of two mechanistic steps: The addition of hydride ions (H^-) on the carbonyl group and protonation of the anion that results in the formation of an alcohol product.



In the first step, the nucleophile (BH_4^-) attacks the carbonyl group. During this attack, it donates H^- to the carbon atom and changes from sp^2 to sp^3 . The nucleophile is the hydride that provides electrons for the formed C-H bond, and the electrophile is the carbonyl group which offers an empty orbital. Therefore, an electron is transferred to the O atom resulting in the C-H bond formation. This interaction indicates that electrons of the π -bond are donated to the oxygen atom, and an alkoxide anion is generated. The second step involves the protonation of the H^+ from the solvent to give the final alcohol product. It has been noted that the production of the alkoxide depends on the polarity of the solvent used. Therefore, reactions are generally carried out in a protic solvent, but it is possible to achieve this in non-protic solvents (Carey and Sundberg, 2007).

However, it is speculated that the alkoxyborohydrides produced can act as reducing agents by successive hydride transfers shown below (Carey and Sundberg, 2007). Thus, the analysis of rate data becomes complicated due to the different routes available.



In spite of this basic understanding of the mechanism, the full details of reducing aldehydes by sodium borohydride remain to be understood. Several theoretical models have been established, although their interpretations conflict, thus clouding the issue.

Early studies by Brown, Mead and Subba Rao investigated suitable solvents for sodium borohydride reductions. It was established that the reaction between aldehydes and ketones follows overall second-order kinetics. The mechanism was considered to proceed by a stepwise hydride ion transfer to the carbonyl carbon, which forms tetraalkoxyborate. This molecule contains the substrate, which is reduced, forming the alcohol product (Brown, Mead and Subba Rao, 1955).

Consequently, they noticed the rate constant was altered in different solvents, demonstrating that solvents play a role in the reaction mechanism. These effects were later re-examined to understand the effects of solvents due to the discovery of iodometric determination of borohydrides (Brown and Ichikawa, 1961). The study reduced acetone in various solvents to determine their effects on the rate constant; these results are summarised below:

Table 2-2: Summary of Solvent Effects on Rate Constants

Solvent	Metal Borohydride	Concentration (M)	Added Material	Concentration (M)	Rate Constant ($10^4 l. mole^{-1} sec^{-1}$)
Water	NaBH ₄	19.40×10^{-3}	-	-	93
Water	NaBH ₄	2.24×10^{-3}	Et ₃ N	0.050	89
Water	NaBH ₄	2.00×10^{-3}	LiCl	0.050	93
Ethanol	NaBH ₄	22.40×10^{-3}	-	-	97
IPA	NaBH ₄	15.9×10^{-3}	-	-	15.1
IPA	NaBH ₄	2.00×10^{-3}	Et ₃ N	0.050	15.2
IPA	NaBH ₄	16.20×10^{-3}	NaI	0.097	15.7
IPA	NaBH ₄	17.80×10^{-3}	LiI	0.010	26.2
IPA	NaBH ₄	17.80×10^{-3}	LiI	0.030	40
IPA	NaBH ₄	17.80×10^{-3}	LiI	0.060	67
IPA	NaBH ₄	17.80×10^{-3}	LiI	0.1	81

The table above shows that a lithium chloride and water solution did not affect the rate; however, in isopropyl alcohol, the rate constant increases by a factor of three to 50.3. Furthermore, increasing the lithium halide concentration increased the rate constant, thus demonstrating that solvents have a vital role in the reaction kinetics.

Houk and co workers devised a model which utilised a transition state geometry model to describe the mechanism of sodium borohydride reductions. The study involved an investigation of cyclohexanones and their remote electrostatic effects. The results make a strong case that the preferential axial attack of hydride to cyclohexanone is caused by torsional strain in the transition state of equatorial attack, and electrostatic effects were also shown to play a role (Wu, Tucker and Houk, 1991). Furthermore, a study investigated the concerted and stepwise pathways of the borohydride reduction of formaldehyde. However, these models do not account for solvent and counterion effects (Eisenstein, Schlegel and Kayser, 1982).

Wigfield suggested an alternative model that considers the role of solvents, yet it does not establish the sodium ion (Na^+) role. These studies assume the solvent alkoxy is contained in the alkoxyborate anion intermediate rather than the product (Wigfield, 1979). Furthermore, isotope studies refuted the initial concept that disproportionation of the intermediate reverts back into BH_4^- (Wigfield and Gowland, 1977).

Several years later, Glass and coworkers demonstrated the critical role of Na^+ by an experimental study on the reduction of carboxylic acids in 2-propanol with NaBH_4^- (Glass, Deardorff and Henegar, 1980). The results showed that sodium ions are coordinated by oxygen atoms found in the alkoxy and carbonyl groups. However, reductions are still possible without Na^+ present. This notion was also substantiated by the work of Yadav, Jeyaraj and Balamurugan (Yadav, Jeyaraj, & Balamurugan, 2000).

The most recent study in 2018 was performed by Patel and co workers. The study established kinetic and mechanistic insights by detailed calculations of the electronic structure of the reduction of indophenol by sodium borohydride. Their work suggested that the reaction occurs in three steps. The first step involves a nucleophilic attack on the carbonyl group forming intermediates. This step is followed by intermediates undergoing proton abstraction assisted by the hydroxyl ion, and the molecule is protonated. Finally, the reduction process is completed by a hydrolysis reaction (Patel *et al.*, 2018).

Despite the conflicting ideas of sodium borohydride reduction, all studies agree that the rate-limiting step is the initial hydride attack. This assumption is based on the

fact that transfers of a hydride ion from relatively weak Lewis acids (alkoxyborines) must be easier than its transfer from stronger Lewis acid borine. This theory is also supported by the potential energy surface constructed by Patel and co workers which describes the system's potential energy. The initial step of reduction is at the peak of (PES) diagram, thus indicating it is the rate-limiting step (Patel *et al.*, 2018).

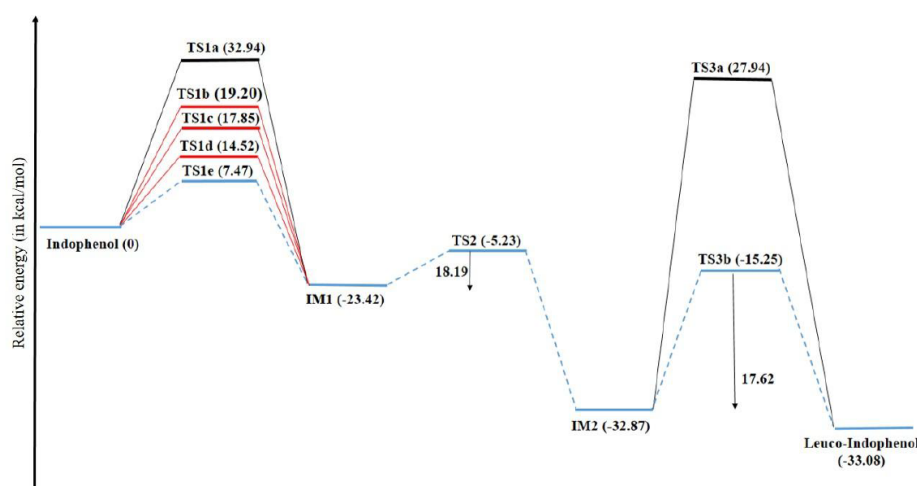


Figure 2-1: Potential energy surface diagram of complete reduction mechanism of indophenol (adapted from *Mechanistic and kinetic insights of reduction of indophenol by sodium borohydride: A theoretical study to explore the effect of solvent and counterion*)

The red lines represent an intermediate reaction, and the black lines depict reduction without a solvent and counterion present. Lastly, the blue lines show solvent and counterion effects. Therefore, the rate law of sodium borohydride reduction should follow a second-order rate law (first order in both the carbonyl compound and borohydride).

2.5. Structure of xylose and potential impacts on reduction

Due to ring chain tautomerism, xylose exists in several forms, including a linear molecule (acyclic form), a six-membered C₅O ring form (pyranose) and a five-membered C₄O ring form (furanose):

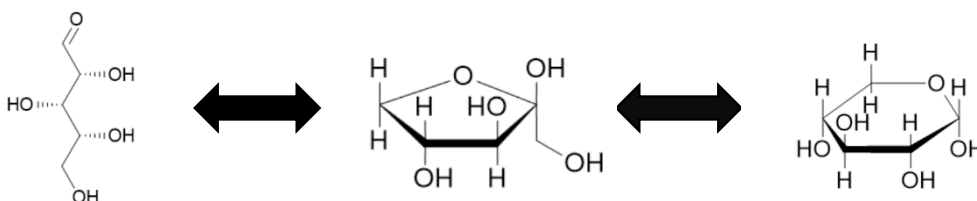


Figure 2-2: Various Structures of Xylose in Solution

The above figure shows that the acyclic structure has an aldehyde group that can react. However, it has been noted that the hemiacetal forms of xylose are most prevalent in solution (Lepilova, Aleeva and Koksharov, 2012). Therefore, it is crucial to understand the conditions which favour the acyclic form of xylose in solution.

A 2012 study conducted by (Lepilova, Aleeva and Koksharov, 2012) looked at these conditions and found that the equilibrium between pyranose and aldehyde forms of monosaccharides can be altered by raising the temperature and the pH of the reaction. These results were based on the presumption that the shift of tautomeric equilibrium toward acyclic structure is the only factor responsible for increasing the reducing power. Therefore, an increase in temperature would improve the conversion of xylose in solution.

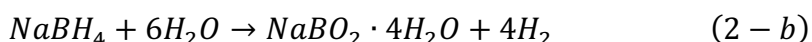
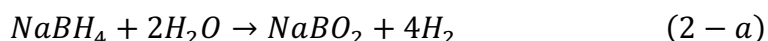
2.6. Solvent effects

As discussed earlier, sodium borohydride reacts in several suitable solvents and plays a crucial role in the reaction mechanism. The following section will look to

understand the reactions that can occur and look at other solvents which are applicable.

2.6.1. Hydrolysis reaction

The hydrolysis reaction between sodium borohydride and water has been studied extensively due to its everyday use as a solvent for reduction reactions and fuel cell operations (Lo, Karan and Davis, 2007). The established reaction pathway is shown below, where two possible reactions can occur:



The two products formed are sodium metaborate and hydrated sodium metaborate, which reduce the reduction capability of sodium borohydride. Gardiner and Collat postulated the reaction mechanism and kinetics, which state the reaction order as pseudo-first-order (Gardiner and Collat, 1965). The reaction is strongly influenced by pH and temperature; a lower temperature and an alkaline solution will retard this reaction (Lyttle, Jensen and Struck, 1952). A significant occurrence during hydrolysis of $NaBH_4$ is that as hydrogen is being released, this will increase the pH and lead to a reduced reaction rate. Furthermore, studies show that an increase in temperature will favour the hydrolysis reaction and reduce the stability of sodium borohydride (Yu and Matthews, 2011). Figures 2-3 illustrate these effects. Furthermore, catalysts such as copper, nickel and cobalt borides and noble metals catalyse the hydrolysis of the borohydride ion (Kaufman and Sen, 1985).

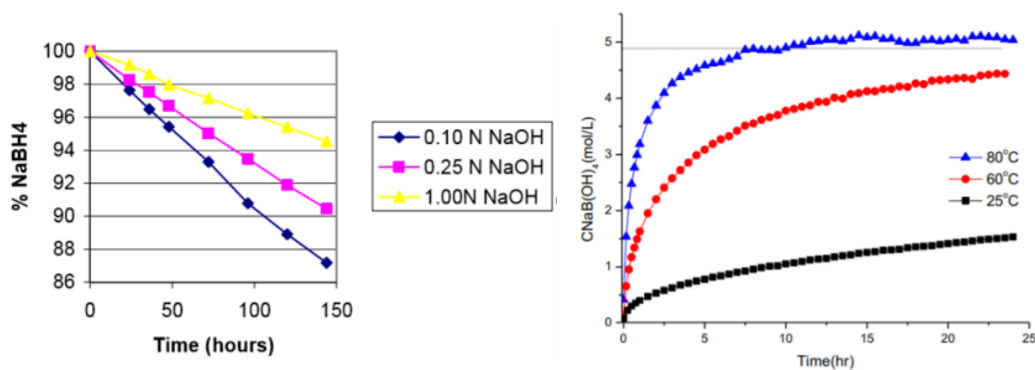


Figure 2-3: (Left) pH effects on Sodium Borohydride (Right) Effects of Temperature on Sodium Borohydride (Adapted from 'Sodium Borohydride Digest')

The following reaction parameters have been modelled to predict the stability of sodium borohydride shown below (Mochalov, Khain and Gil'manshin, 1965).

$$\log_{10} t_{\frac{1}{2}} = pH - (0.034T - 1.92) \quad (2 - c)$$

Where the $t_{1/2}$ represents the half-life in minutes, and T is the temperature (Kelvins). This equation will be used to determine the pH of the sodium borohydride solution discussed in the methodology.

2.6.2. Solubility of sodium borohydride in water

The work by Jensen measured the solubility of sodium borohydride in water at different temperatures, and the data is illustrated in graph 2-4. Furthermore, Appendix A describes the solubility of sodium borohydride in various solvents. This table shows that sodium borohydride is generally soluble in most polar solvents that contain an amine or hydroxyl group (Yamamoto, 2003). The graph shows the equilibrium temperature of the two forms, NaBH₄ and NaBH₄•2H₂O, both in crystal form. Two curves are present, and the one below 36.4 describes the solubility of the dihydrate while the curve above 36.4 represents the solubility of anhydrous NaBH₄ (Jensen, 1954).

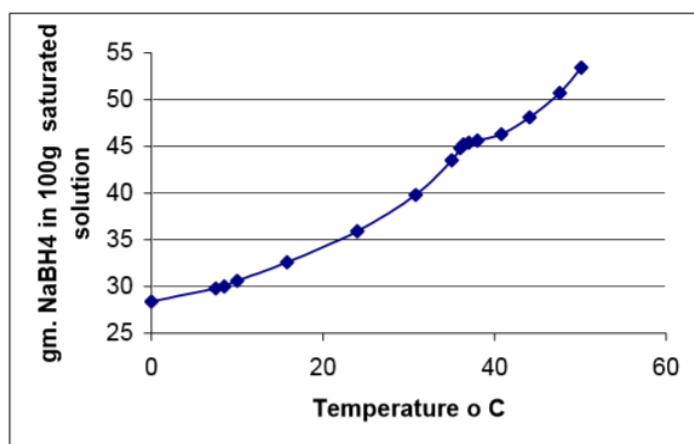


Figure 2-4: Solubility of Sodium Borohydride in Water

From graph 2-4, we can see that with time, sodium borohydride's solubility in water increases with increasing temperature, making it a commonly utilised solvent.

2.6.3. Suitable solvents

As mentioned before, the stability of sodium borohydride is related to the extent of hydrolysis, which can occur. Sodium borohydride has shown to be unstable in primary alcohols with low molecular weights (e.g. methanol, ethanol and ethylene glycol) as well as acidic alcohols (e.g. phenol). However, it has been shown that adding a base to the low molecular weight alcohol causes it to be more stable. The work conducted by Rohm and Haas further supports this. The experiment added 0.01N sodium methoxide to a methanol solution and added sodium ethoxide to an ethanol solution. Their results have shown that the solvolysis reaction is suppressed when bases are present in the solution (Yamamoto, 2003).

Other solvents such as N-methyl-2-pyrrolidone, dimethylacetamide, pyridine, di and triethylene glycol and dioxane are suitable solvents in the sense that there is no chance of hydrolysis. However, these solvents can be dangerous and have been shown to slow down reduction reactions. Such as the case with dimethylformamide

(DMF), where an exothermic reaction can occur. Therefore, using alkaline water as a solvent to determine the reaction kinetics is practical due to its widespread use, cost, and safety.

2.7. Xylitol production

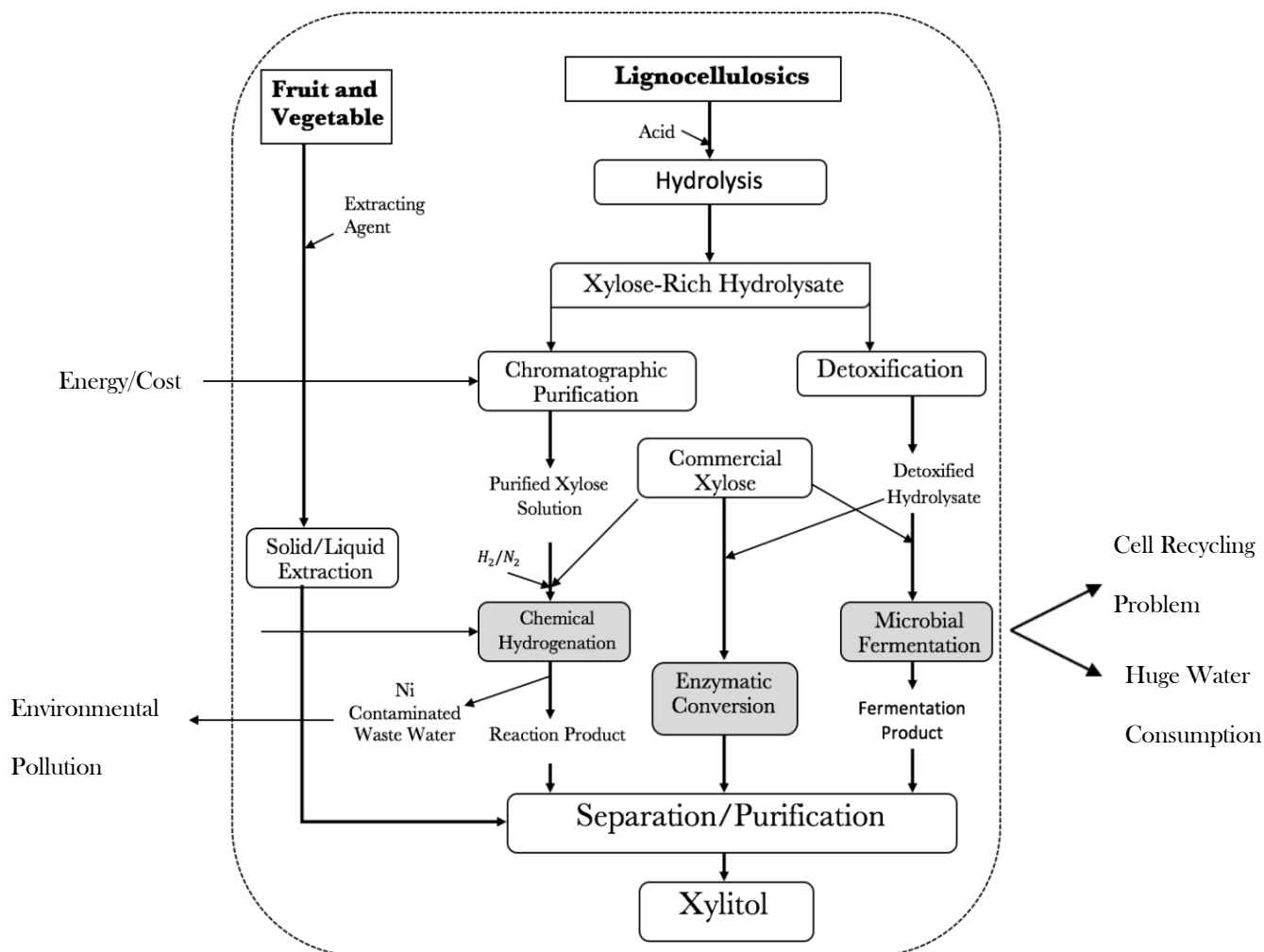


Figure 2-5: Overview of Xylitol Production Techniques

2.7.1. Catalytic hydrogenation

Conventional methods of producing xylitol involve the hydrogenation of pure xylose, which typically uses a three-phase slurry batch reactor in the presence of a Raney nickel catalyst. The typical reaction operating temperature and pressure range from (80-140°C) and (1-50atm) respectively (Chen *et al.*, 2010). The choice

of utilising a nickel catalyst is due to its ease of use as a suspended slurry in batch reactions, activity, selectivity and lower price (Mishra, Dabbawala and Hwang, 2013). However, Raney Ni catalysts have relatively fast deactivation due to organic impurities accumulating on the catalyst surface. This process causes poisoning of active sites and metal leaching (Gallezot *et al.*, 1998). As a result, the process selectivity and conversion rates are limited.

Furthermore, to use xylitol in the food industry, it is essential to remove all impurities through several purification steps, including ion exchange, filtering, and crystallisation (Mikkola *et al.*, 1999). Xylitol yield is about 50-60% of the xylan fraction of 8-15% of the starting raw material. Thus, production through the hydrogenation process is a technically complicated multistep process with relatively low efficiency, making it costly (Rafiqul and Sakinah, 2013). Therefore, alternative chemical production techniques have been researched, such as a well-known method involving protection group chemistry or producing a pentanol from a hexaldonic acid such as gluconic acid. However, due to a lack of commercial interest and different starting compounds, these methods are not considered adequately at an industrial level (Sheldon and Bekkum, 2008).

2.7.2. Enzymatic routes

Enzyme technology is an alternative and promising production technique for producing xylitol. These methods provide an environmentally friendly and low-cost route to produce xylitol from hemicellulosic hydrolysates. One mole of the coenzyme is utilised per mole of xylitol produced because the XR-mediated biohydrogenation of xylose is NAD(P)H dependent. Several studies on the

enzymatic route have been conducted with the notable results summarised in Table 2-3:

Table 2-3: Review of Reported Enzymatic Production Techniques

Species Examined	Conditions	Results Obtained	Comments	Reference
Candida pelliculosa with oxireductase system of <i>Methanobacterium sp.</i>	-Temperature: 35°C -pH of 7.5 -24 hour period	Yields: 90%	-	(Kitpreechavanich <i>et al.</i> , 1984)
Candida tenuis coupled with glucose dehydrogenase from <i>Bacillus cereus</i>	-150 hour reaction time with a single dose of NADH	Yields: 96% Productivity: 3.33g/L.h	Extensive NADH dependent activity, long-term stability and absence of strong product inhibition	(Nidetzky <i>et al.</i> , 1996)
Candida tenuis coupled with formate dehydrogenase from <i>C. boidinii</i>	-pH of 7 -Temperature: 25°C -Total Reaction Volume: 20mL -25mM Tris/HCL buffer	Fed-batch conversion of xylose to xylitol: 2.8g/L.h from 0.5M xylose	An improvement as results of 3 times greater than conversion achieved in conventional batch reaction	(Neuhauser <i>et al.</i> , 1998)
Candida peltata within electrochemical bioreactor containing graphite-Mn(IV) electrode	-Temperature: 30°C -pH of 6.5	Yield: 65%	The cathode acts as a catalyst for the reduction of NAD ⁺ to NADH. Comparatively, an improvement on classical bioreactors	(Park <i>et al.</i> , 2005)

Optimisation of XR-catalysed reactions is crucial for their performance in the production of xylitol synthesis. However, current literature shows that this technique is still not commercially competitive due to process bottlenecks; only a selected number of microorganisms can significantly reduce xylose, hindering optimisation space (Buijs, Siewers and Nielsen, 2013). Additionally, glucose present in many microorganisms represses xylose metabolism through carbon catabolite repression (Park, Vinuselvi and Lee, 2012). Consequently, this leads to poor xylose conversion efficiency coupled with high reaction times. To ensure the feasibility of this process, it is essential to extensively study several parameters

affecting productivity. These parameters include temperature, pH, and the initial concentrations of the substrate and coenzyme.

2.7.3. Microbiological routes

Since the expenses related to conventional chemical production and in vitro enzyme-based manufacturing are high, more economical and environmentally sustainable production methods for xylitol have received attention. One of these processes is the biosynthesis of d-xylose using microorganisms. The microbiological process uses bacteria, fungi and recombinant strains to produce xylitol from pure xylose or a hemicellulosic hydrolysate.

Bacterial production of xylitol. The conversion of d-xylose to d-xylulose in bacteria occurs in a single step and is catalysed by xylose isomerase. Several researchers have conducted screening experiments to determine the xylitol production yield from different species. A study conducted by Rangaswamy and Agblevor found that out of the 17 cultures screened, the bacteria that produced the highest amount of xylitol was *Corynebacterium sp. B-4247* (Rangaswamy & Agblevor, 2002). Another study conducted by Izumori and Tuzaki assessed 420 bacterial strains based on their capacity to produce xylitol from d-arabinitol. Their findings showed that *Gluconobacter oxydans* were the best xylitol producer with a yield of 29.2 g/L xylitol from 52.4g/L d-arabinitol after an incubation period of 27 hours (Izumori & Tuzaki, 1988). However, owing to the relatively low amount of xylitol produced, it has caused researchers to lose interest in xylose-fermenting bacteria.

Fungal production of xylitol. The reaction mechanism for most fungi and yeasts requires a reduction step followed by an oxidation step. In this reaction mechanism,

d-xylose was first reduced to d-xylitol by either NADH- or NADPH-dependent xylose reductase. The resultant d-xylitol was either further oxidised to d-xylulose or secreted. In d-xylose fermentation and d-xylitol production, these two reactions are considered the rate-limiting steps (Chen *et al.*, 2010). This production technique has been studied to a lesser extent relative to other biotechnological production techniques. Several filamentous fungi such as *Aspergillus*, *Byssoschlamys*, *Gliocladium*, *Penicillium*, *Rhizopus* and *Neurospora* sp. have shown the ability to produce xylitol. However, they produce small quantities, and there has only been one significant study on this topic (Dahiya, 1991). The report involved using *Petromyces albertensis* to produce xylitol, with a yield of 0.4 g/g from 100g/L-d-xylose. Additionally, studies on *Fusarium oxysporum* and *Mucor* sp. produced negligible amounts of xylitol (Ueng and Gong, 1982).

Therefore, it is evident that biotechnological routes of producing xylitol are not ready to compete with chemical routes currently in place. However, reducing xylose using sodium borohydride can provide an intermediary process for xylitol production, minimising the detrimental effects of hydrogenation techniques.

2.8. Conclusion

This review aims to outline the critical understanding of sodium borohydride reductions and assess the shortcomings of previous work. Reductions in sodium borohydride have been utilised for almost a century. However, apparent disparities in certain mechanistic features persist. Despite disagreements, the reaction rate has been established to follow an overall second-order reaction, with solvents and cations playing critical roles in the reaction rate. Furthermore, certain structural features of xylose and xylitol have been outlined to understand how reaction

parameters affect xylitol production. Finally, to assess the feasibility of the production of sodium borohydride, it is crucial to establish the various techniques currently being implemented and researched in the space of xylitol production. The findings show that although biotechnological production techniques provide significant benefits over chemical routes, several bottlenecks prevent these techniques from being implemented on an industrial scale. Therefore, making sodium borohydride a viable intermediary solution for xylitol production.

2.9. References

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Chapter 3: Design of experiments

3.1 Introduction

The following quantitative study was performed to determine the kinetics of the reaction between xylose and sodium borohydride. The two primary goals of kinetic studies are to determine the reaction order of xylose and gain insight into each elementary reaction within the overall reaction (i.e. the reaction mechanism). The basic technique of determining rate constants is to initiate a reaction and monitor the successive changes in concentration of reagents as a function of time using appropriate analysis techniques. Monitoring methods are dictated by the reaction timescales (which can vary from hours to femtoseconds) and therefore, the first step is establishing the correct reaction timescale. Additional aspects of understanding include the stability of the reactants to determine temperature ranges and monitor the reaction, concentration effects, and conversion. This chapter details the experimental methods/materials and diagnostics utilised in the experiments performed. Detailed information regarding the sample preparation procedures, raw materials used, conditions and characteristic techniques are shown. Designing the experiment required understanding all conventional methods utilised in kinetic experiments and applying suitable methods for the experimental trials. Furthermore, the methods of analysing the results obtained are included.

3.2 Research design

An experiment is conducted in which purposeful variations in controlled input variables (X_n) are made to observe and determine causes in the output response (Y_n) (Figure 3-1). However, there are no feasible factors to control in most cases, either due to the expense or physical limitations. These are termed disturbance or noise

factors that influence the response. However, several strategies exist that can determine the factors and the interactions between them that have on the output response.

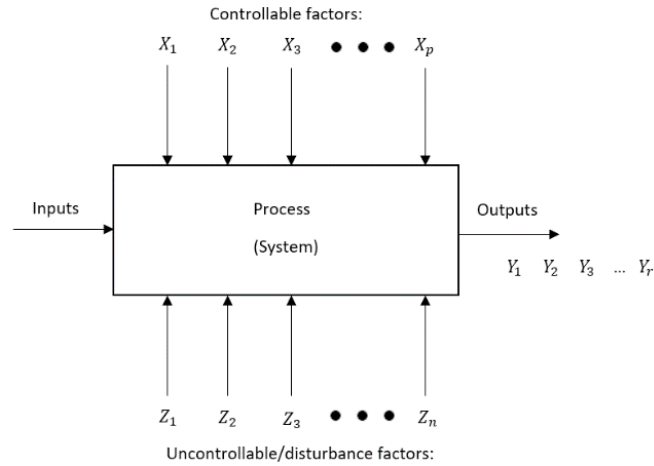


Figure 3-1: Experimental Variables in Design of Experiments

The optimum approach to dealing with several factors is to conduct a factorial experiment. Other methods include the best guess approach or the one-factor-at-a-time approach (OFAT), yet both have proven time-consuming and costly (Eriksson *et al.*, 2008). The factorial design adjusts factors together rather than one at a time, and further details will be discussed. There are three different experimental objectives that the design achieves. The first is in screening, where the most influential factors are determined, and the functional design space or range is defined. The other is to investigate the combination of factors that lead to the optimal experimental objective. The final is robustness testing which involves determining how sensitive the product/process is to minor variations in each factor. This technique is valuable as it maximises the information output while simultaneously minimising the resources required for experiments. Therefore, DoE

is an effective tool to understand the reaction parameters for reducing xylose using sodium borohydride.

3.2.1 Response surface methodology

A type of experimental design is the response surface methodology (RSM) and is the foundation of factorial designs. The goal of RSM is to optimise the response of interest through mathematical and statistical techniques. These techniques utilise modelling and analysis in cases where several variables influence the response of interest. The mathematical representation of the relationship between the response and the input variable levels is shown below (Box and Draper, 2006):

$$y = f(x_1, x_2, \dots, x_k) + \epsilon \quad (3 - a)$$

Where ϵ represents the error or noise represented by :

$$E(y) = f(x_1, x_2, \dots, x_k) = \eta \quad (3 - b)$$

The surface is modelled by these equations, which is termed the response surface. This technique can be represented graphically in a contour plot as seen in Figure 3-2, where η is plotted against levels of $x_1, x_2, \dots, x_k$ which are the unknowns. In these contour plots, constant response lines are drawn in $x_1, x_2, \dots, x_k$ plane, and each contour corresponds to the height of the response surface. The relationship between the independent variable and the response is unknown in several cases of RSM. Therefore, the initial phase of RSM involves seeking a suitable approximation for y and the set of independent variables. When a response follows a linear function of the independent variable, the following model approximates the first-order model.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k + \epsilon \quad (3 - c)$$

However, when the system has observed curvature, a higher-degree polynomial should be adopted, as represented in the following equation.

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i < j} \sum \beta_{ij} x_i x_j + \epsilon \quad (3 - d)$$

Between these two functions, almost all RSM cases can be utilised to form appropriate models. It is unlikely that a polynomial function can model the entire space of the independent variables.

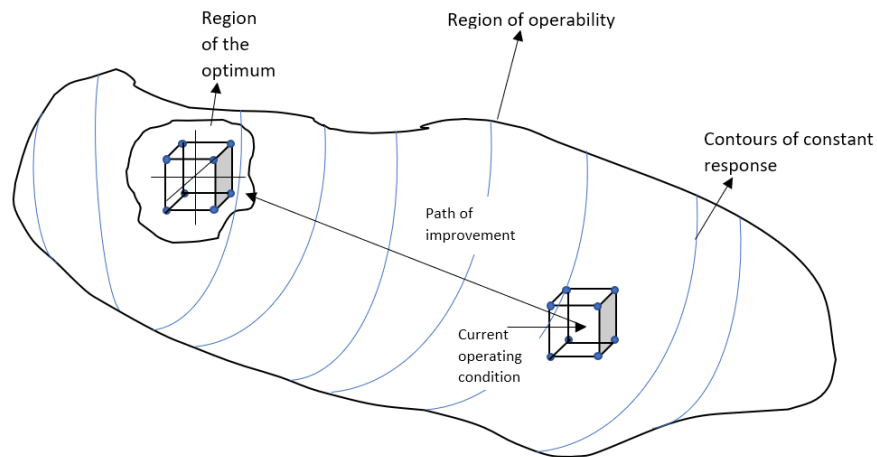


Figure 3-2: Contour Plot of General RSM Procedure

Often in RSM, when the current operating conditions show slight curvature in the system, it can be considered remote from the optimum. However, over a relatively small region, they can approximate these variables sufficiently. Once the optimum region has been found, a second-order model can be utilised together with analysis to find the optimum.

RSM has been employed in several studies to optimise, screen and produce prediction models of reaction parameters in xylitol production from eligible yeasts.

Sampaio and co workers applied RSM to optimise xylitol production from *Debaryomyces hansenii* UFV-170, a xylitol-producing yeast. Independent variables were rotational speed, initial xylose concentration biomass concentration, and xylitol yield. Volumetric productivity, maximum xylitol concentration and specific productivity were chosen as response variables. The optimal range of the independent variables where responses were optimised was conducted using RSM. This investigation provided an optimal range of independent variables where all responses are simultaneously optimised (Sampaio *et al.*, 2006). Silva and Roberto conducted a 22 full-factorial central composite design to determine the combined effects of initial xylose concentration and inoculum level on xylitol production by *Candida guilliermondii*. RSM allowed for a mathematical model to predict process variables and showed validity compared to experimental results (Silva and Roberto, 2001). Rodrigues *et al.* utilised RSM as in the bioconversion of xylose into xylitol by *Candida guilliermondii* FTI 20037. The process variables chosen were temperature, pH, activated charcoal pre/post the process and xylose concentration. A 2^{5-1} factorial central composite design was used, and results showed a fivefold increase in initial xylitol concentration (Rodrigues *et al.*, 2003). Lastly, optimisation of culture mediums and process variables for xylitol production using corncob hemicellulose hydrozylate by *Pachysolen tannophilus* (MTTC 1077) was reported by Ramesh *et al.* Nine nutrients were screened using a Plackett-Burnam design and the selected nutrient conditions were optimised using a central composite design. This analysis established a significant prediction model that showed consistent experimental results and provided an enhanced xylitol yield

(Ramesh *et al.*, 2013). Therefore, RSM is an appropriate tool in factorial designs to determine optimal operating conditions.

3.2.2 Factorial design methodology

Factorial designs permit the simultaneous study of the effects that several factors may have on a process. The effects mentioned are measured by how the response varies when one or more factors are changed. This design assesses the most influential factors on the response and the effect of interactions between these factors. This feature is essential as complex processes commonly have synergistic and antagonistic interactions between input variables. Furthermore, compared to the traditional one factor at a time approach, it requires fewer trials and provides more information. A simple experimental design is the two-factor factorial design, where each factor is assigned two levels. The two possible levels assigned are high (+) and low (-) to construct an orthogonal array of experiments. Orthogonal experimental designs have zero correlation between any variable or interaction effects specifically to avoid this problem (Anderson and Whitcomb, 2016). Therefore, regression results for each effect are independent of all other effects, and the results are clear and conclusive. Factorial designs conduct the analysis assuming a linear relationship between two factors, and a test should be included to test for curvature between factors to avoid misleading predictions. Furthermore, replicate centre points were included in the design of the experiment to ensure an accurate empirical model. In a case where k factors are investigated, 2^k measurements (excluding centre points) are required to perform a factorial design analysis. The following study chose initial xylose concentration, temperature and reaction time as the independent variables. The agitation rate was fixed at 800 rpm, and the ratio

of sodium borohydride was kept constant. Table 3-1 below summarises the levels for each operating factor. The ranges selected for factors are based on initial trials and work conducted by (Abdek-Akher, Hamilton and Smith, 1951) and (Heikkilä, et al., 1999).

Temperature	Concentration	Time
Degrees	mol/L	Seconds
Numeric	Numeric	Numeric
25 (-)	0,4 (-)	50 (-)
60 (+)	1 (+)	300 (+)
42.5 (C.P)	0.7 (C.P)	175 (C.P)

Two-level factorial designs provide a solid foundation for further experiments. Original factorial designs can be augmented with axial points in the response surface design, optimising the response with greater precision. Furthermore, an initial factorial design can guide the current design in a different design space according to the steepest ascent/descent path. Additionally, a better understanding of the highest potential region can be done by conducting a second factorial design with fewer variables and a smaller design space.

For these reasons, a factorial design was used to study the main effects and the interaction effects between significant operational parameters in the batch reaction to produce xylitol from xylose and sodium borohydride. The details of this experimental design can be seen in Appendix 3-A. However, the main drawback of this experimental technique is that the size of the design is a function of the examined factors, which can lead to an excessive number of trials. Therefore, only three key factors were selected in the experimental procedure: temperature, time, and xylose concentration.

3.3 Kinetic analysis and determination of rate constants

The following section describes methods to determine critical aspects relating to the reaction kinetics. These include establishing appropriate reaction techniques and methods for determining the reaction rate constant and the reaction order.

3.3.1 Kinetic analysis techniques

Results obtained from initial testing show that the reaction has a half-life between 30 and 50 seconds and can be classified as a slow to moderately fast reaction. Therefore, according to Figure 3-6, classical methods are the recommended monitoring methods. Three methods considered were flow experiments, stopped-flow techniques and batch mixing:

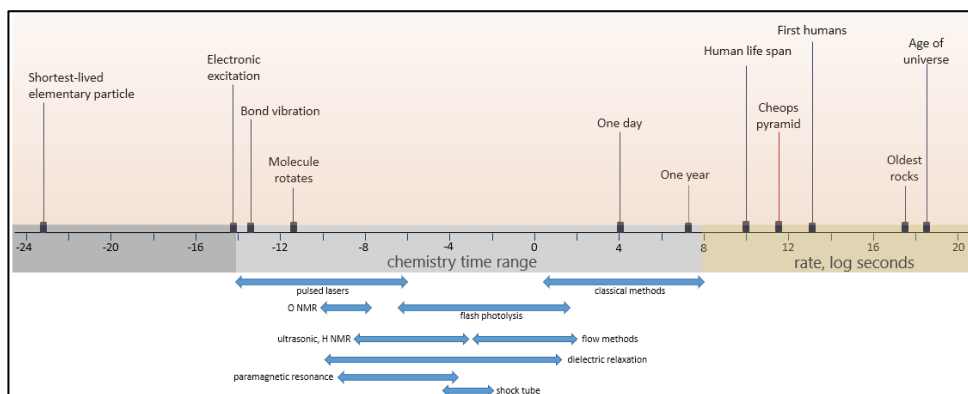


Figure 3-3: Time Scale of Reactions

Flow experiments involve reactants being fed to a reactor and react flowing in a pipe that allows for on-line/in situ monitoring. Stopped flow techniques are utilised in reactions that occur within milliseconds. This method rapidly mixes reactants in a specialised vessel and flows to the detection cell. Reactants are rapidly mixed in a mixing chamber, and the flow is stopped with the reactant stream in the flow cell.

Batch mixing experiments is the simplest of the three methods. The method involves removing samples at set intervals and termination using quenching techniques appropriate for the reaction. Stir-in-pot reactions are monitored by removing samples at set intervals, and the reaction is terminated using specific quenching techniques. The techniques involve manipulating experimental conditions; for example, chemical quenching involves adding a new reagent to the reaction. In many cases, reactions are sensitive to pH and can be quenched with the addition of a base or acid. Freeze quenching involves dropping the temperature rapidly, and optical quenching occurs when the light of a specific wavelength is exposed to the sample. This technique was implemented in the study as it provides the most straightforward method without requiring specialised equipment. Furthermore, this method follows experiments that have been conducted in a similar method (Nygren *et al.*, 1997).

3.3.2 Reactions carried out under pseudo-order conditions

The reaction order can be determined by employing the empirical rate equations utilising pseudo-first-order conditions. Experiments were conducted by keeping sodium borohydride in excess while altering the concentration of xylose.

$$Rate = k[Xylose]^a[NaBH_4]^b \quad [NaBH_4] \gg [Xylose] \quad (3 - e)$$

During the reaction, $NaBH_4$ concentration will remain unchanged. This simplification will allow the equation to determine the pseudo-order rate law of xylose.

$$Rate = k[Xylose]^a \quad (3 - f)$$

The new rate constant k' is dependent on the value of the overall rate of k and the initial concentration of sodium borohydride. Hence the only reactant concentration that varies is xylose. This approach simplifies the experimental procedure and the subsequent calculation.

3.3.3 Initial-rate method

The method of initial rates involves initiating the reaction multiple times under controlled conditions and measuring the reaction rate in each run. Variables are held constant in each run; however, the concentration of one reactant is varied and repeated until all reactants concentration is adjusted. This method allows the rate law to be determined by observing how reaction rates vary as each reactant concentration is altered. The rate law can be used to determine the value of the reaction rate constant (k) for each trial. This method can be helpful where a reaction is too slow to follow or complicating side reactions. The following equations describe the methodology mathematically:

$$v_0 = k_f[A]_0 \qquad k_f = \frac{v_0}{\Delta[A]_0} \qquad (3 - g)$$

The initial rate (v_0) for the reaction, which includes reactant A giving a product P. The value of k_f is determined by dividing the initial rate by the change in reagent concentration ($\Delta[A]_0$). Plotting the concentration of product [P] against time will give a line where the gradient is $k[A]_0$. This simple method will determine the kinetic order from the initial rate from different initial reagent concentrations. Although this method is simple in practice, it only measures a portion of the reaction and does not give adequate information on how the reaction progresses, which is

imperative in understanding the feasibility of the reaction between xylose and sodium borohydride.

Non-linear Regression Analysis measures concentrations as a function of time, comparing their time dependence with the appropriate integrated rate laws. The isolation method discussed earlier is employed, simplifying the rate law to determine its dependence on a single reactant's concentration: (Fogler, 2016). Therefore, this simplified rate is substituted into the integrated rate law as follows:

$$\frac{dCa}{dt} = -kCa^a \quad (3-h)$$

$$Ca_0^{1-a} - Ca^{1-a} = (1-a)kt \quad (3-i)$$

$$Ca = [Ca_0^{1-a} - (1-a)kt]^{\frac{1}{1-a}} \quad (3-j)$$

$$s^2 = \sum_{i=1}^N (Ca_{mi} - Ca_{ci})^2 = \sum_{i=1}^N [Ca_{mi} - [Ca_0^{1-a} - (1-a)kt_i]^{\frac{1}{1-a}}]^2 \quad (3-k)$$

The purpose of non-linear regression is to seek values of a and k that minimise S , the sum of the squares of the deviations of the calculated xylose or xylose concentration values from the observed ones. As discussed in the results section, xylose concentration data will be used to determine the reaction order. Non-linear regression is an iterative process; thus, suitable software such as Minitab is used to conduct the regression analysis (Lee and Yoo, 2018; Arminian and Ozgur, 2020). The main advantage of using such software packages is the possibility of fitting non-linear curves to the data, including those requiring complex equations. The

fitting method selected was the least-squares method which minimises the sum of squares of the vertical distance between data points and the curve.

The initial values are selected for each parameter and then repeatedly changes those values to improve the 'goodness of fit'. The parameter range is stipulated in table 4 and was selected as reaction order must range between 0-3. The reaction rate constant k value ranges between 0,0001 and 1 because an adverse reaction rate would imply the reaction is reversed. Regression terminates when varying parameter values results in negligible changes in the goodness of fit. If a solution provides convergence, it does not guarantee that the model fit is optimal or that the sum of squared errors (SSE) is minimised. The non-linear fitting of data does not compromise the statistics as data is not linearised. However, mathematical functions are selected by the user and fitted to the data and can lead to misleading assumptions if not correctly applied. The kinetic analysis depends on accurate experimental data, and no manipulation will extract reliable results from poor data sets. Furthermore, it is essential to understand that the size of the dataset is a crucial parameter to consider. Reducing the dataset can considerably change rate constants even if the rate order remains the same.

3.3.4 Arrhenius equation

The Arrhenius equation is a function that represents the fraction of collisions that have the required energy to overcome the activation energy. This energy is termed activation energy E_a and can be seen below. The following equation is used to understand how the reaction rate is related to the temperature at which it is conducted.

$$k = Ae^{-\frac{Ea}{RT}} \quad (3 - l)$$

The pre-exponential function is the frequency of collisions between reactant molecules as standard concentrations. This term is the fraction of molecules that would react if the activation energy were zero or if the kinetic energy of all molecules exceeds the activation energy.

$$\ln k = \ln A - \frac{Ea}{RT} \quad (3 - m)$$

Rewriting the Arrhenius equation in a non-exponential form provides a convenient method to interpret results graphically. The plot $\ln k$ vs $(1/T)$ yields a straight line; thus, estimating the activation energy at only two temperatures is convenient. The following set of equations show how to determine activation energy through solving simultaneous equations.

$$\ln k_2 - \ln k_1 = \left(\ln A - \frac{Ea}{RT_2} \right) - \left(\ln A - \frac{Ea}{RT_1} \right) \quad (3 - n)$$

$$= \frac{Ea}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \quad (3 - o)$$

$$Ea = \frac{R \ln \frac{k_2}{k_1}}{\frac{1}{T_1} - \frac{1}{T_2}} \quad (3 - p)$$

The following method was used to solve the activation energy for the reaction between xylose and sodium borohydride, and the results are presented in the next chapter.

3.4 Analytical methods for xylose/xylitol characterization

Carbohydrates are typically challenging compounds to analyse due to their complex nature. These complexities are due to the carbohydrate chains and multiple linkage points present in the structure. Additionally, most carbohydrates, including xylitol and xylose, lack chromophore and fluorophore moieties, making separation more challenging. Therefore, it is essential to review suitable analysis techniques to separate, analyse and characterise these molecules accurately.

3.4.1 HPLC/HPAEC-PAD methods

High-performance liquid chromatography (HPLC) is an analytical tool that separates complex mixtures with high sensitivity. This technique is a superior form of column chromatography because the solvent is pumped under high pressure of up to 400 atmospheres, making the technique more rapid than column chromatography. HPLC can be combined with a variety of detection units based on the compound being analysed. The ideal detector must have adequate sensitivity, good stability and reproducibility, linear response, and be non-destructive. Refractive Index detector and ELS detectors have proven suitable for detecting xylitol since it lacks chromophoric and fluorophic moieties (Bhandari *et al.*, 2008; Cheng, Chen and Hsieh, 2010).

Several developments in HPLC have led to the discovery of a chromatographic technique named high pH anion exchange chromatography (HPAEC). This method has allowed the detection of carbohydrates in various complex matrices such as in foods, beverages and dairy products. This method provides highly selective separation as it exploits the weakly acidic nature of carbohydrates. Coupled with a pulsed amperometric detector (PAD), it allows direct quantification of non-

derivatised carbohydrates at low picomole levels with minimal sample preparation and clean-up. Both HPAEC and PAD need high pH mobile phases to allow the chromatographic separation of the anionic sugars through ion exchange mechanisms (Martínez Montero *et al.*, 2004).

Anion exchange chromatography is a form of ion-exchange chromatography (IEX). IEX takes advantage of molecules net surface charge to cause separations. Specifically, HPAEC takes a positively charged ion exchange resin with an affinity for molecules having a net negative surface charge. Dealing with carbohydrates that are weak acids (pKa values ranging between 12-14) and, at high pH, are converted to oxyanions readily separated using highly efficient anion-exchange columns. Separation in HPAEC columns occurs according to structural features such as size, composition, anomericity and linkage isomerism.

The detection unit, pulsed amperometry, is an extension of single-potential amperometry. Two electrodes are positioned in the column effluent, and a voltage is applied and measured. The current varies at the anode and cathode as the electroactive analyte is oxidised/reduced. The current is measured while the potential is applied only, and the detector processes sequential measurements to provide smoother results. PAD is typically implemented when analytes foul the electrode, reducing the signal for every analysis. Furthermore, it provides a more selective and suitable response when compared to other detection methods such as mass spectrometry. Therefore, the following technique is the most suitable and is applied in the experimental study.

3.4.2 Pyrolysis gas chromatography

Analytical pyrolysis is a powerful technique that allows for rapid analysis of complex and heterogeneous organic materials. This method is based on controlled thermal degradation of the sample in an inert atmosphere. The organic matter is either flash vaporised (whether thermally stable and volatile) or degraded to lighter neutral molecules. There are three stages in pyrolysis: initiation, propagation and termination. The crucial steps involved are the rapid temperature increase of the sample and the fast removal of products with inert gas from the heated part of the equipment, as it avoids secondary reactions and allows for optimal degradation. The benefit of this technique is that it requires a minute amount of sample (<1mg) and can provide information on an extensive range of organic materials that are difficult to detect through other analytical procedures. Identification of degradation products is acquired through coupling pyrolysis with gas chromatography and mass spectrometry (Py-GC-MS) which provide retention times and mass spectra (Montalbani, 2012).

A study conducted by Huang and co workers investigated xylose's thermal degradation reaction mechanisms using density functional theory methods. They proposed that during pyrolysis, there were eight possible pyrolytic reaction pathways. Furthermore, it was shown that the significant xylose products pyrolysis is low molecular products such as glycolaldehyde 5, methylglyoxal 7, acetaldehyde 11, 2-furaldehyde 14, acetone 21 and CO, and the main competitive products are formaldehyde, formic acid, acetic acid 30, CO₂, CH₄, acetol 35, 5-hydroxy-pyran-4-one 38, 4-hydroxy-pyran-2-one 40 (Huang *et al.*, 2016).

Another study conducted by Räisänen *et al.* assessed the thermochemical behaviour of sugars (D- and DL-arabinose, D- and DL-xylose and D-mannose) as well as a sugar alcohol (D- and DL-arabinitol) using pyrolysis-gas chromatography with mass-selective detection (Py-GC/MSD).

Table 3-1: Thermal degradation compounds of polyols and pentose sugars

	Retention Time (min)	Degradation Compound
D-Arabinose / D-Xylose	4.8	(2H)-furan-3-one
	5.9	2-furancarboxaldehyde
	6.6	furanmethanol
	8.6	1-(2-furanyl)-ethanone
	9.1	α -angelicalactone
	12.1	3-hydroxy-2-penteno-1,5-lactone
	13.1	3-methyl-1,2-cyclopentanedione
	15.3	methyl ester of furoic acid
	28.8	tricyclo[6.2.0.0(3,6)]dec-1(8)-en-2,7-
	41.7	hexadecanoic acid
D-Xylitol / Arabinitol	4.9	(2H)-furan-3-one
	5.9	2-furancarboxaldehyde
	6.7	furanmethanol
	9.1	α -angelicalacton
	12.0	3-hydroxy-2-penteno-1,5-lactone
	13.1	3-methyl-1,2-cyclopentanedione
	22.8	1,6-anhydro- β -D-glucopyranose
	41.7	hexadecanoic acid

In each case, the primary pyrolysis products were classified into the following compound groups: (i) furanes, (ii) pyrenes, (iii) cyclopentane, (iv) cyclohexanes, (v) anhydroglucopyranose, (vi) dianhydroglucopyranose, and (vii) saturated fatty acids. The prominent peaks of the chromatograms of pentoses (arabinose, xylose) and sugar alcohols (arabinitols) were different. The most significant peak of pentoses was 2-furancarboxaldehyde, and hexose was (2H)-furan-3-one. The highest peak of arabitol at pyrolysis temperature of 500°C was furan methanol and

550°C α -angeligalactone. Table 3-3 summarises the main components found in pentose and sugar alcohols (Räisänen *et al.*, 2003). The following method, in conjunction with HPAEC-PAD, was utilised to analyse the samples.

3.4.3 Capillary electrophoresis method:

Capillary electrophoresis is an analytical technique that separates ions based on electrophoretic mobility along with an applied voltage. This technique has been developed for trace analysis due to the ability to analyse sizes from nanolitres to femtolitres and the short analysis time. The fast analysis time is achieved by applying high voltages, and separation is not dependent on column length. Feasible detectors that can be used are fluorescence and mass spectrometry, as well as electrochemical detection. Methods established by Yuan and Dong in 2016 have been shown to successfully detect xylitol along with a variety of alditols in food samples. These techniques showed a fast and straightforward analysis with a detection limit of <0.1 g/L and 3mg/L and were completed within 11 minutes.

Furthermore, studies have shown that capillary electrophoresis successfully detected acidic, neutral amino sugars and sugar alcohols (including xylose and xylitol). This method used indirect UV detection using 2,6-pyridine dicarboxylic acid as an electrolyte. Thus this method would be appropriate for achieving the experimental objective of quantifying xylose and xylitol simultaneously (Soga and Heiger, 1998). However, it was not implemented as Py-GC-MS and HPAEC-PAD provide superior analysis according to the literature (Yuan and Dong, 2016).

3.5 Methodology

The following section specifies the experimental procedure and the materials used. The literature in the preceding sections influenced the details of the procedure.

3.5.1 Raw materials

The following provides specifications of the raw materials that were used during the experimental procedure:

Table 3-2: Summary of Raw Materials

Chemical	Formula	Molecular Weight	Manufacturer
Xylose	$C_5H_{10}O_5$	150.13 g/mol	Sigma Aldrich
Sodium Borohydride	$NaBH_4$	37.83 g/mol	Merck
Sodium Hydroxide	$NaOH$	39.99 g/mol	Sigma Aldrich
6N Hydrochloric Acid	HCl	36.46 g/mol	Sigma Aldrich

3.6 Experimental procedure

Figure 3-7 is a representation of the experimental setup. Predetermined xylose and sodium borohydride solutions were added to the three-neck round bottom flask and the dropping funnel, respectively. Table 3-5 shows the initial concentration for xylose and sodium borohydride. The mols of sodium borohydride were measured to be roughly three times the amount of xylose to meet the assumption for the method of excess. The three-neck round bottom flask is placed within a glycerol bath to ensure even heating of the solution. This bath was placed on a hot plate combined with a magnetic stirrer providing heat and agitation to the reaction solution. The hot plate has a temperature probe that measures the reaction temperature and adjusts according to the set temperature. The role of the reflux condenser is to cool down generated vapours and convert them back into a liquid form, which prevents loss of reaction solution.

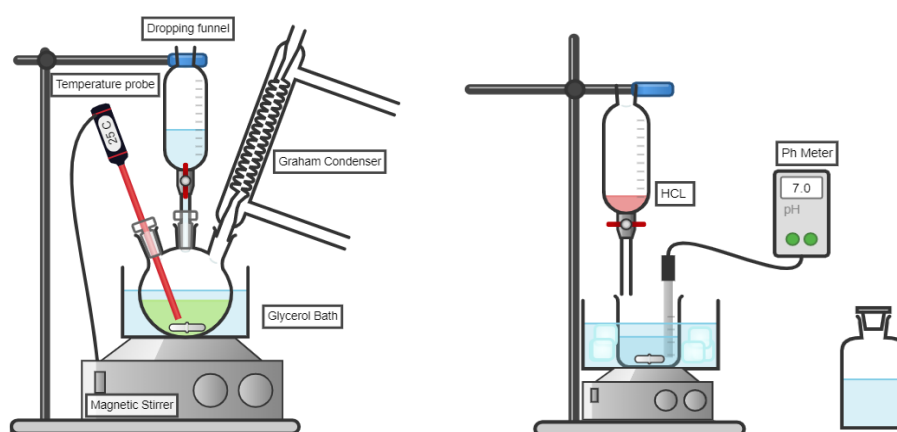


Figure 3-4: Graphical Representation of Experimental Setup

The magnetic stirrer is switched on, and the temperature and RPM knobs are used to regulate the variables accordingly. Cold water from the tap can flow through the condenser to ensure no vapours escape and mitigate product loss. Once the temperature of the xylose solution has stabilised at a set temperature, the sodium borohydride is then added in a dropwise manner.

Table 3-3: Summary of Experimental Conditions for Kinetic Experiments

Experimental Conditions	
Temperatures	[25C; 60C]
Agitation Speed	800 RPM
Initial Concentrations	
[Xylose]= 0.4 ± 0.05 mol/L	[NaBH ₄]= 0.28 ± 0.03 mol/L

Over one-minute intervals, 10ml solution was removed and then quenched by placing it in an ice bath, and 5ml of hydrochloric acid was added. Figure 3-7 represents how the acid was added to the sample. A pH meter was connected to ensure the solution is acidic enough to decompose excess sodium borohydride. The solution is then sealed in a plastic container and refrigerated. Solutions were then prepared for HPAEC-PAD and Py-GC-MS analysis. Preparation for HPAEC-PAD

analysis included diluting the sample with 10 ml of deionised water. The samples are then weighed and underwent vacuum filtration. Two methods of preparation for Py-GC-MS were conducted to remove moisture in the samples. The first involved oven-heating the solution at 105 °C, and the second involved freeze-drying the samples. Following this, the samples were weighed and analysed.

3.7 Conclusion

The following study seeks to establish the reaction kinetics while assessing specific reaction parameters regarding the reduction of xylose. To do so, a batch reaction approach was employed to collect concentration data for both xylose and xylitol over 5 minutes. Data collection was based on both the integral and the method of excess to provide pseudo-order rate laws, and the thermal properties of the reaction were calculated according to the Arrhenius equation. Analysis of this data is performed utilising non-linear regression techniques and is discussed further in subsequent chapters.

The second set of experiments involved a 2^3 factorial design establishing an empirical model to predict the conversion and yield of xylose and xylitol. This method provides an economical method that maximises the information gained from the experiment with centre points to establish optimal reaction conditions. Both experiments included replicate points to ensure accuracy and improve time resolution.

Furthermore, samples were analysed using High-Performance Anion Exchange Chromatography coupled with a pulsed amperometric detector. According to the

literature outlined in this chapter, HPAEC-PAD has proven to be the most effective in detecting the complex structures of carbohydrates.

3.8 References

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Chapter 4: Experimental Results

4.1 Introduction

The study aims to provide more information regarding the reaction kinetics between xylose and sodium borohydride and assess the effects of temperature, time, and concentration on the reaction over specified ranges. Certain aspects were discussed in the previous chapter, where the methodology of analysis was presented. In this chapter, the experimental results are presented and statically analysed to verify their validity concerning the study.

The factorial design involved 20 separate trials with two replicates, and the kinetic experiment consisted of 2 batches with three replicates. Furthermore, initial attempts used pyrolysis GC-MS which proved to be unsuccessful. The pyrograms are represented in Appendix 4C. Nonlinear regression and the excess method were used to provide the reaction order and reaction rate constants as outlined in Section 3.3.2.

Replication of both experiments measured the reliability of results. Concentration data for xylitol did not show any sense of reliability with the erratic patterns, and potential reasons will be discussed. However, xylose concentration data showed similar repeated results. Thus the study focused on analysing xylose concentration data to provide kinetic information and an empirical model for conversion over specified ranges.

Stat-Ease Design-Expert software was used to conduct the factorial design analysis, and Minitab was used in the nonlinear regression analysis; these results will be presented in the following chapter.

4.2 Kinetic Study

4.2.1 Summary of Results

Figures 4-1- 4-6 include graphs representing the successive changes of xylitol and xylose concentration over a 5-minute interval. The dotted red line represents this theoretical limit, and any point which exceeds this line cannot be considered scientifically accurate. A similar issue found in the factorial design can be witnessed in these results. The amount of xylitol produced exceeds the stoichiometric limit. Nearly all points in the replicate data exceed this and are disregarded. Hence, there is insufficient data to conduct a kinetic analysis using xylitol concentrations (Morrison, 2021). Troubleshooting this issue included repeated analysis of samples. Nonetheless, results did not differ, implying decay of xylitol or xylose after months of refrigeration, and no issues can be linked to the analysis procedure.

The graphs included in 4-7 are graphical representations of all xylose replicates at different temperatures. The error analysis is shown in Section 4.2.2. of the xylose replicates provide sufficient error that makes them suitable for analysis. Xylose concentration data indicates a more gradual rate of concentration change at lower temperatures compared to higher temperatures. Additionally, batch 2 reacted fully within 225 seconds, and the conversion in batch 1 reached 81.47% after 5 min. These observations imply that the reaction rate constant should be greater in batch 2. These observations will be further discussed in the following chapter.

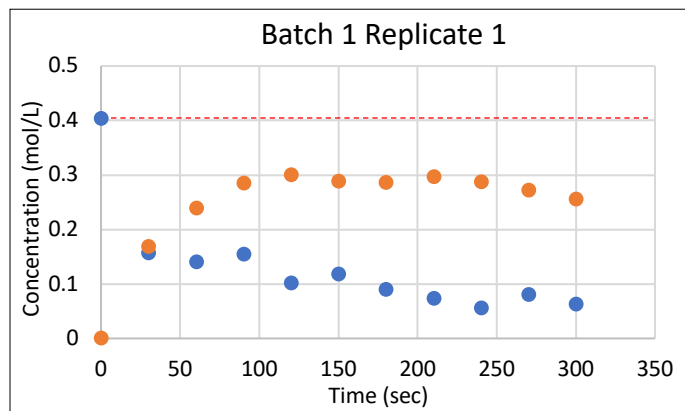


Figure 4-1: Concentration Data for Batch 1 (Replicate 1)

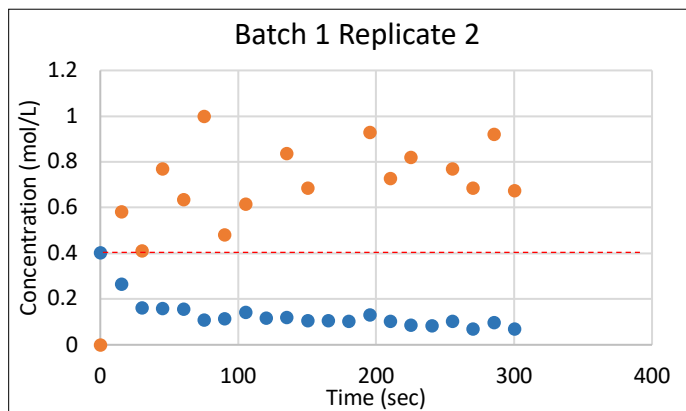


Figure 4-2: Concentration Data for Batch 1 (Replicate 2)

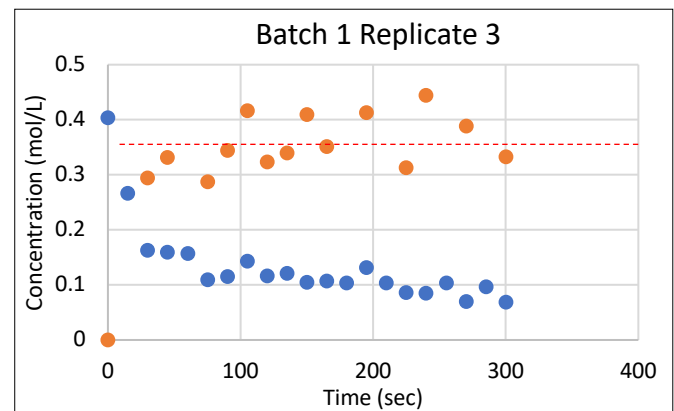


Figure 4-3: Concentration Data for Batch 1 (Replicate 3)

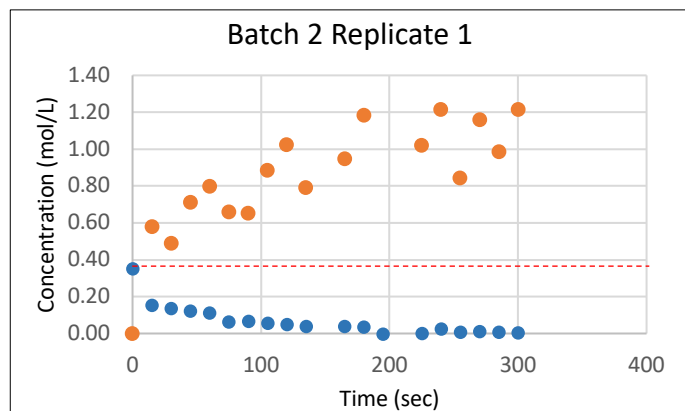


Figure 4-4: Concentration Data for Batch 1 (Replicate 1)

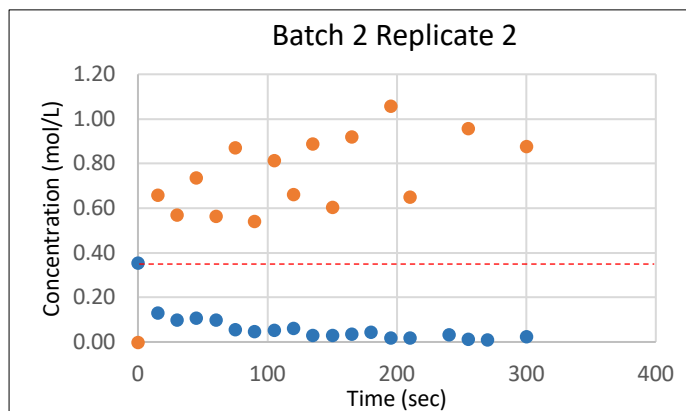


Figure 4-5: Concentration Data for Batch 2 (Replicate 2)

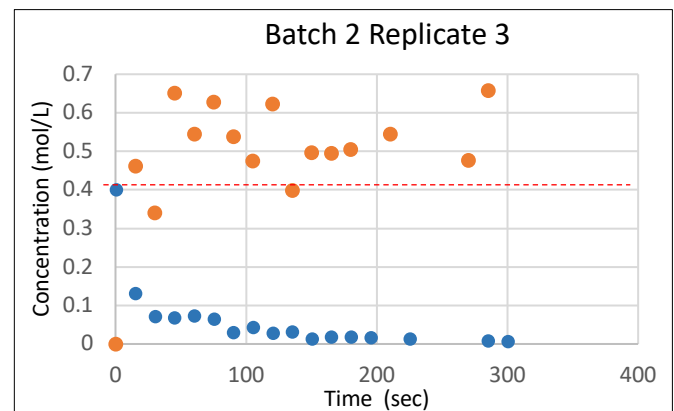


Figure 4-6: Concentration Data for Batch 2 (Replicate 3)

4.2.2 Xylose replicate results

The error is a combined measure of the inherent variation in the phenomenon we observe and the numerous factors that interfere with the measurement. Every effort should be made to reduce systematic errors through efforts such as calibrating measurement instruments. It is impossible to eliminate all measurement errors. If the underlying error is truly random (not biased), we can still gain valuable information by making multiple observations (i.e. replicates) and calculating the average. These calculations are shown in Appendix 4A.

$$\bar{x} = [\sum_{i=1}^n x_i]/n \quad (4 - a)$$

$$s^2 = \sum_{i=1}^n (x_i - \bar{x})^2 / (n - 1) \quad (4 - b)$$

$$s_{\bar{x}} = s/\sqrt{n} \quad (4 - c)$$

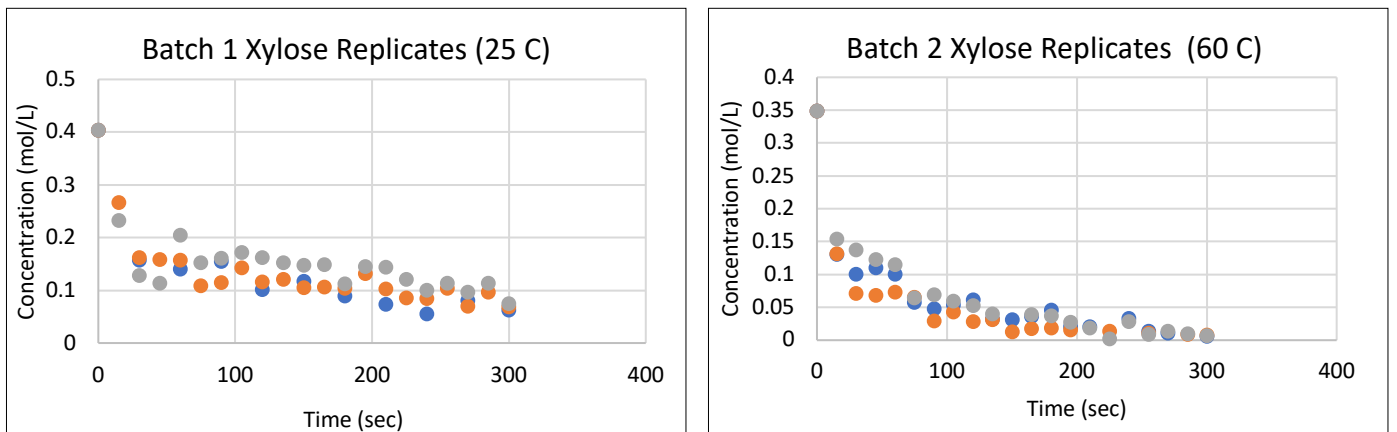


Figure 4-7: Xylose Replicate Data for Batch 1 and 2

4.2.3 Analysis of kinetic data

Integrated rate laws are utilised to express the reaction rate as a function of the initial concentration and a measured concentration of one or more reactants after a specific amount of time (t) has elapsed; they are used to determine the rate constant and the reaction order from experimental data. Measured concentrations as a function of time allow for comparing their time dependence with the appropriate integrated rate laws. The isolation method discussed in section 3.3.2 is employed, which is a technique for simplifying the rate law to determine its dependence on a single reactant's concentration. The dependence of the reaction rate on the chosen reactant concentration is isolated by having all other reactants present in excess so that their concentration remains nearly constant throughout the reaction, as indicated in the following equation set (Fogler, 2016).

The purpose of nonlinear regression is to seek values of a and k that minimise S , the sum of the squares of the deviations of the calculated xylose or xylose concentration values from the observed ones. As discussed in the results section, xylose concentration data will be used to determine the reaction order. Nonlinear regression is an iterative process; thus, Minitab software is utilised to determine the reaction order (Lee and Yoo, 2018; Arminian and Ozgur, 2020). The fitting method selected was the least-squares method, which minimises the sum of squares of the vertical distance between data points and the curve. The initial values are selected for each parameter, and then those values are repeatedly changed to improve the 'goodness of fit'.

Table 4-1: Summary of Key Results in NLR Analysis:

Starting Values		Parameter Estimates	
Parameter	Values	Estimate	95% CI
Batch 1			
<i>a</i>	0-3	1.25	(0.74 ; 1.68)
<i>k</i>	1×10^{-4} -1	9.76×10^{-3}	$(4 \times 10^{-3}; 2.17 \times 10^{-3})$
Batch 2			
<i>a</i>	0-3	1.37	(1.19; 1.42)
<i>k</i>	1×10^{-4} -1	3.44×10^{-3}	$(2.47 \times 10^{-2}; 4.18 \times 10^{-2})$

The parameter range is stipulated in Table 4 and was selected as reaction order must range between 0-3. The reaction rate constant *k* value ranges between 1×10^{-4} , and 1 because a negative value would imply the reaction is reversed. Regression terminates when varying parameter values result in negligible changes in the goodness of fit. If a solution provides convergence, it does not guarantee that the model fit is optimal or that the sum of squared errors (SSE) is minimised. Convergence on incorrect parameter values can occur due to a local SSE minimum or an incorrect expectation function (Meloun and Militký, 2011). Therefore, it is crucial to examine and ensure the parameter values, fitted line plots, and residual plots are reasonable. Minitab provides output data that assess the model fit. Minitab's estimates show that the reaction order is 1.37 and 1.25 for batch 1 and 2, respectively. The estimates for the reaction rate constants are 3.44×10^{-3} and 9.76×10^{-3} in batch 1 and 2. Tables 4-2 and 4-3 summarise these assessments.

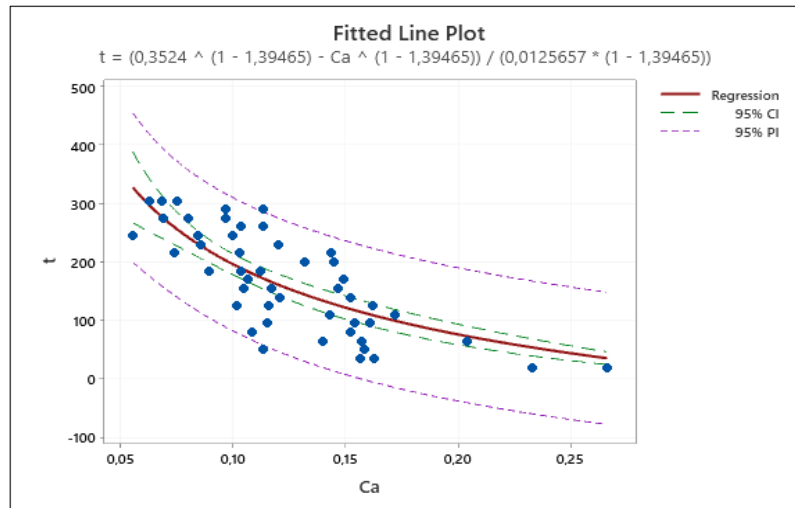


Figure 4-8: Fitted Line Plot (Batch 1)

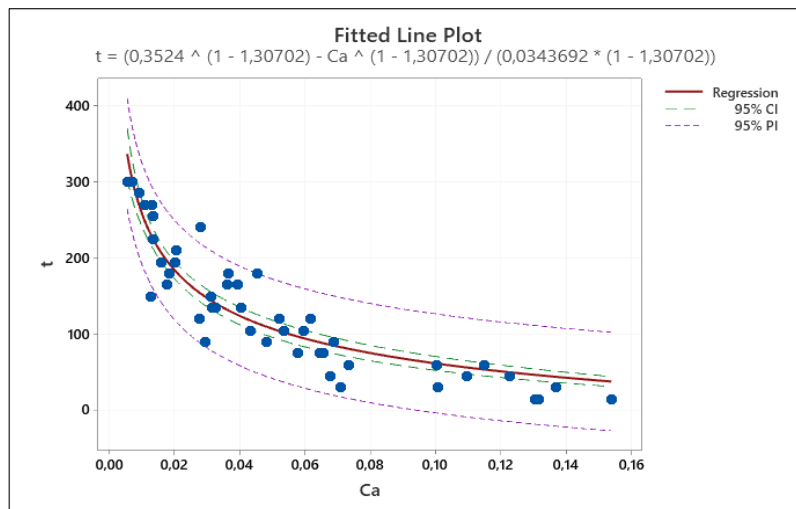


Figure 4-9: Fitted Line Plot (Batch 2)

The regression line (bold red) follows the curvature in the points relatively closely, and only 2 points show significant deviation from the fitted line. These points are at 0.027 and 0.044 and can be attributed to experimental error. The points adequately cover the entire range of predictor values. The 95% confidence interval (CI) is stipulated in these tables, representing the likelihood (95% confidence) of the results lying within the interval. The CI can be seen graphically as the dotted green line in Figures 4-8 and 4-9. The prediction interval (PI) for the fit provides a

range of probable values that will fall within the interval with 95% confidence indicated by the purple lines (Motulsky and Christopoulos, 2004).

Minitab's ANOVA analysis gives the p-values for batch 1 and 2, respectively, are 0.78 and 0.99. A comparison between p values and significance level is required to ascertain whether the model appropriately postulates the relationship between the response and the predictors. This test assesses the null hypothesis, indicating no association between the term and the response. A significance level of 0.05 is generally chosen, indicating a 5% risk of concluding that a false association exists. Therefore, the p-value is greater than the significance level; hence the test does not detect a significant lack of fit. Please note that the R^2 indicator is not a valid tool to assess the goodness of fit because the sum of error variance and explained variance does not explain the total variance (Spiess and Neumeyer, 2010).

Table 4-2: Table for Lack of Fit Data

Source	DF	SS	MS	F	P
Batch 1					
Error	47	1310246	27878		
Lack of Fit	46	1116009	24261	0.12	0.78
Pure Error	1	194237	194237		
Batch 2					
Error	47	1310246	27878		
Lack of Fit	46	1116009	24261	0.12	0.99
Pure Error	1	194237	194237		

The total degrees of freedom (DF) is the number of sample observations used to estimate the unknown population parameters. The analysis uses that information to estimate the values of unknown population parameters. The DFE found in Table 4-3 represents the DF of error subtracted by the DF of pure error. The different sums

of squares (SS) for error calculate the variance attributable to the total error, lack of fit error, and pure error. This is calculated by the difference between the sum of squares for error and the sum of squares for pure error. To determine the amount of variation assigned to the total, lack of fit and pure error, the different mean squares (MS) is used. The mean squares are calculated by the sums of squares divided by their degrees of freedom. The MSE found in Table 4-3 is the different mean squares and is calculated by dividing the final SSE by the DFE. Finally, the F-value represents the Lack of Fit term. This value is used as a test to assess whether a model is missing higher-order terms which contain predictors. A relatively large F-value generally means the lack of fit is significant. These terms are utilised to calculate the p-value, which is used to assess the lack of fit.

Table 4-3: Summary of NLR data

	Batch 1	Batch 2
Iterations	7	18
Final SSE	47088	146851
DFE	46	47
MSE	1023.66	3124.49
S	31.99	55.90

The standard error of the regression (S) represents how far the data values fell from the fitted values and is used to determine the model's efficiency in describing the response. S is measured in the units of the response variable. A low value of S implies the model describes the response well and indicates that the observations are closer to the fitted line. The S values calculated are 31.99 and 55.90, which are large, yet these were the lowest S obtained values over this range. However, a low S value does not indicate that the model meets the model assumptions, and residual plots must verify the assumptions.

4.2.4 Nonlinear regression residual analysis

The residuals versus fits plots are assessed to verify that the residuals are randomly distributed and have constant variance. Figures 4-11 and 4-13 represent the "residual versus fits plot"; this is a scatter plot used in the residual analysis. It is a scatter plot of residuals on the y-axis and fitted values (estimated responses) on the x-axis. The residuals are randomly scattered around the x-axis on both figures and form a rough horizontal band. This pattern supports the assumption that the relationship is linear and the variance of error terms is equal. Furthermore, no one residual deviates significantly from the residuals' basic pattern, which suggests no outliers.

Figures 4-10 and 4-12 display the residuals versus order plots for batch 1 and 2. The plot is used to support the claim that the residuals are independent of one another. Patterns such as a cycle or shift imply that residuals close to each other may be correlated and not independent. Independent residuals show no trends or patterns when displayed in time order. In this case, time predictors should be added to the model. Furthermore, residuals exhibiting normal random noise around the residual x-axis suggest no serial correlation. The residual versus order plot illustrated exhibits no significant patterns, and therefore, we can conclude that residuals are not correlated (Martin, De Adana and Asuero, 2017).

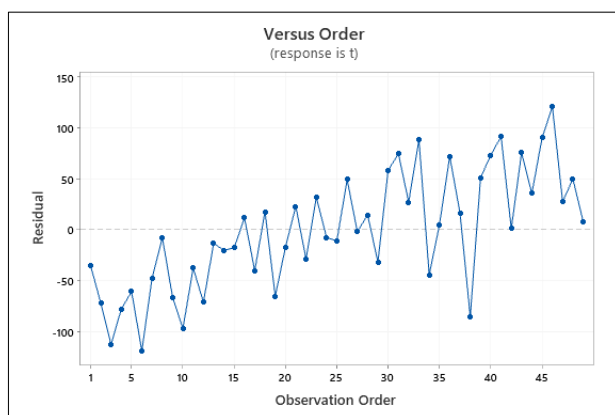


Figure 4-10:Residual vs Order Plot (Batch 1)

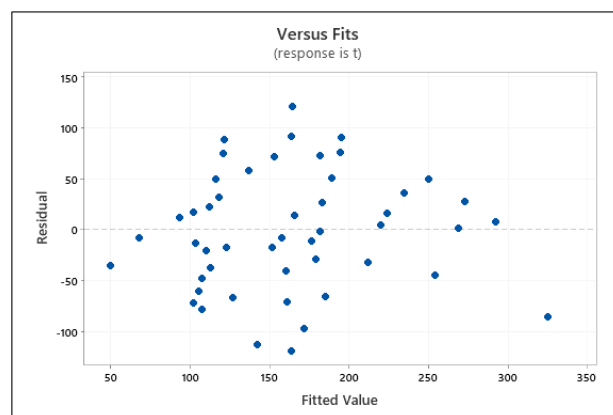


Figure 4-11:Residual vs Fits Plot (Batch 1)

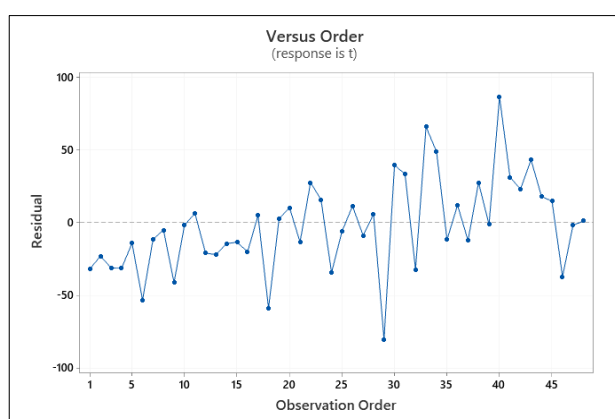


Figure 4-12:Residual vs Order Plot (Batch 2)

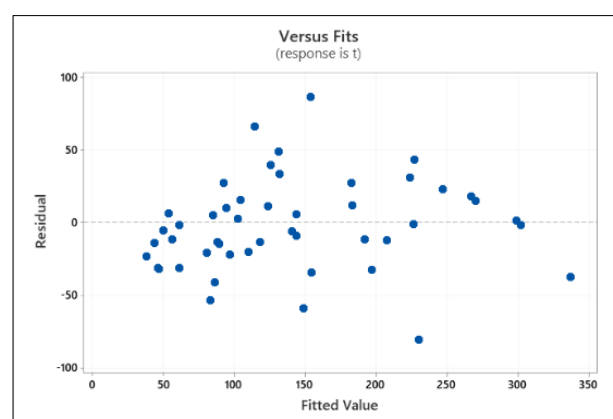


Figure 4-13:Residual vs Fits Plot (Batch 2)

4.3 Arrhenius equation

The Arrhenius equation is used to determine the activation energy (E_a) and pre-exponential factor (A) shown in Equations 6 and 7. A simple calculation from temperatures of 25°C and 60°C determined the value for the E_a and A to be 31.74 kJ/mol and 19146.72 respectively.

4.4 Initial pyrolysis–gas chromatography–mass spectrometry

Appendix 4C represent pyrograms from initial trials. These observations show that both xylose and xylitol are fragmented, making it difficult to track their concentrations. Samples were freeze-dried rather than oven-dried during sample

preparation to troubleshoot this problem. The main degradation compounds were analysed as a reference to identify xylitol and xylose, which are summarised below.

Table 4-4: Main Degradation Compounds in Oven-Dried and Freeze-Dried Samples

Oven-Dried degradation compounds	Freeze-Dried degradation compounds
Furancarboxaldehyde	2-Furanmethanol
Formic Acid	Formic Acid
Furan 2-methyl	Furan 2-methyl
1-Butanol	2-Butenal
Furan 2,5 dimethyl	
Acetic acid	
2- Pentanone, 4-hydroxy-4methyl	

However, both xylose and xylitol share the same degradation compounds, as seen in Table 3-3. Therefore, pyrograms were compared to find patterns and identify increasing and decreasing peaks in each batch. However, out of 80 trials, only 3 detected xylitol as a peak. Out of the 80 samples which ran under Py-GC/MS, only three pyrograms provided peaks of xylitol. These samples were pure freeze-dried samples (2.1-2.3). However, a loose pattern is present in the main degradation compounds (acetic acid, furancarboxaldehyde). This pattern shows an initial increase in concentration from 1.1 to 1.3 followed by a significant decrease in 1.4 or 1.3 with a steep increase to 1.5. These results support the mechanistic theory that causes this erratic behaviour. However, similar degradation compounds make it difficult to make any assertions on the reaction kinetics.

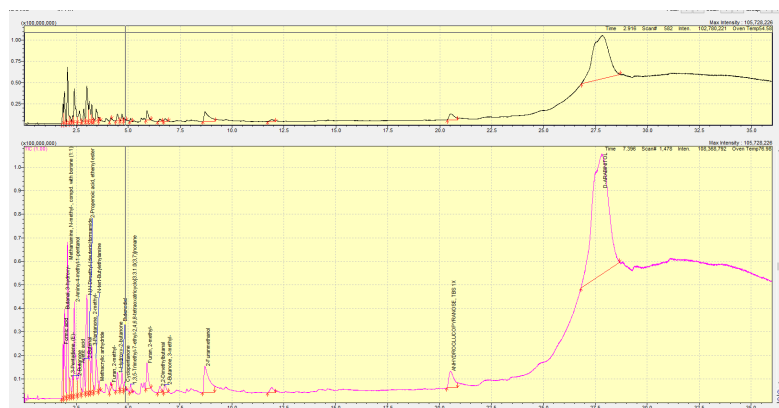


Figure 4-14: Pyrogram of Commercial Xylitol

(xylose/xylitol) has been violated. In runs where the stoichiometric law was not broken, the maximum yield found was 73.33% in test 6 with a conversion of 98.88%.

Table 4-4: Results of Factorial Design

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	A: Temperature	B: Concentration	C: Time	Yield	Conversion
	°C	mol/L	s		
4	25	0.4	50	0.76	0.48
18	25	0.4	50	0.73	0.58
7	60	0.4	50	1.13	0.77
11	60	0.4	50	1.11	0.66
2	25	0.4	300	1.63	0.79
14	25	0.4	300	1.79	0.77
1	60	0.4	300	3.05	0.98
5	60	0.4	300	2.79	0.98
9	42.5	0.7	175	0.67	0.85
12	42.5	0.7	175	0.58	0.89
19	42.5	0.7	175	0.64	0.82
10	25	1	50	0.53	0.75
16	25	1	50	0.57	0.75
15	60	1	50	0.58	0.92
20	60	1	50	0.69	0.84
13	25	1	300	0.66	0.93
17	25	1	300	0.74	0.93
6	60	1	300	0.73	0.99
8	60	1	300	0.73	0.98

Trials 4 and 18 show that the yields obtained were greater than those of test 6. However, it should be noted that run 4 was significantly milder compared to run 6 and had significantly lower conversions. Considering the yield obtained and the conditions under which they were performed, all highlighted xylitol results were disregarded. Hence, the study focused on analysing the kinetics from xylose data and determining conversion rather than xylitol yield.

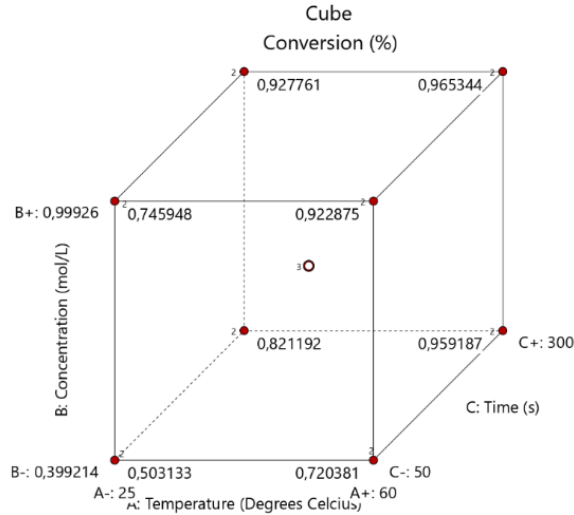


Figure 4-16: Cubic Plot of Factorial Design

4.5.2 Analysis of factorial design

The model coefficients were estimated using Stat-Ease Design-Expert software.

This empirical model is represented below:

$$(y)^3 = -0.20 + 2.47 \times 10^{-3}(x_1) + 0.24(x_2) + 8.29 \times 10^{-4}(x_3) + 1.46 \times 10^{-3}(x_2)(x_3) - 6.80 \times 10^{-5}(x_1)(x_2)(x_3) \quad (4 - d)$$

The analysis of variance (ANOVA) results, along with the fit results, are tabulated in Table 4-2. Similarly, in nonlinear regression, the model p-value is $< 1 \times 10^{-4}$, verifying that it is statistically significant. The p-value for curvature in the ANOVA summary was given as 0.76. Thus, it is statistically not significant (defined as < 0.05), providing evidence of a nonlinear relationship between at least one factor and the response.

The coefficient of determination (R-Squared) is 0.93, which explains the variation observed in the response that the selected model explains. The term 'Adj R-Squared' is 0.87, the R-Squared adjusted for the number of model terms, and the value

decreases as the number of unnecessary terms is included in the model. The 'Pred R-Squared' value is 0.74, which measures the model's predictive capability for future values.

Table 4-5: Summary of ANOVA Results and Fit Results

ANOVA Results					
Source	Sum of Squares	df	Mean	F-value	p-value
Model	1.08	7	0.15	27.72	$< 1 \times 10^{-4}$
A-	0.27	1	0.27	47.99	$< 1 \times 10^{-4}$
B-	0.23	1	0.23	40.36	$< 1 \times 10^{-4}$
C-Time	0.50	1	0.50	89.90	$< 1 \times 10^{-4}$
AB	2.3×10^{-3}	1	2.3×10^{-3}	0.42	0.53
AC	8×10^{-3}	1	$8. \times 10^{-3}$	1.44	0.26
BC	4.58×10^{-2}	1	4.58×10^{-2}	8.20	1.69×10^{-3}
ABC	3.19×10^{-2}	1	3.19×10^{-2}	5.71	3.8×10^{-3}
Curvature	1.23×10^{-2}	1	1.23×10^{-2}	2.21	0.17
Pure Error	5.59×10^{-2}	10	5.6×10^{-3}		
Cor Total	1.15	18			
Fit Results					
R²	0.95				
Adjusted R²	0.92				
Predicted R²	0.83				
Adeq Precision	14.80				

Tests for normally distributed residuals and outliers were conducted to ensure model assumptions have not been violated. These plots are represented in Figures 4-3. The residual vs run plot illustrates Figure 4-3(a) tests the response for lurking variables that influenced the experiment. Any trends in this plot would imply that time-related variables are present. This plot illustrates a random scatter, therefore, proving lurking variables do not exist. Figure 4-3 plots represent the residuals vs ascending predicted response values and are a graphical test for constant variance. Furthermore, Figure 4-3(c) represents the normal probability plot, where the normal distribution is represented by the straight red line on the plot. Initially, the normal

probability plot showed an S-shaped curve, and the plot displayed a megaphone pattern. These trends suggest a case of expanding variance, and a transform is required to satisfy the assumption of homogeneous variance. Most cases use power transformations. Therefore, the Box-Cox transformation method was used to test for the optimal value of λ ; in this case, it is 3 (Box and Cox, 1964). The updated residual plots are shown in Figure 4-3, which shows a random distribution of the residuals, indicating constant variance (Anderson and Whitcomb, 2007).

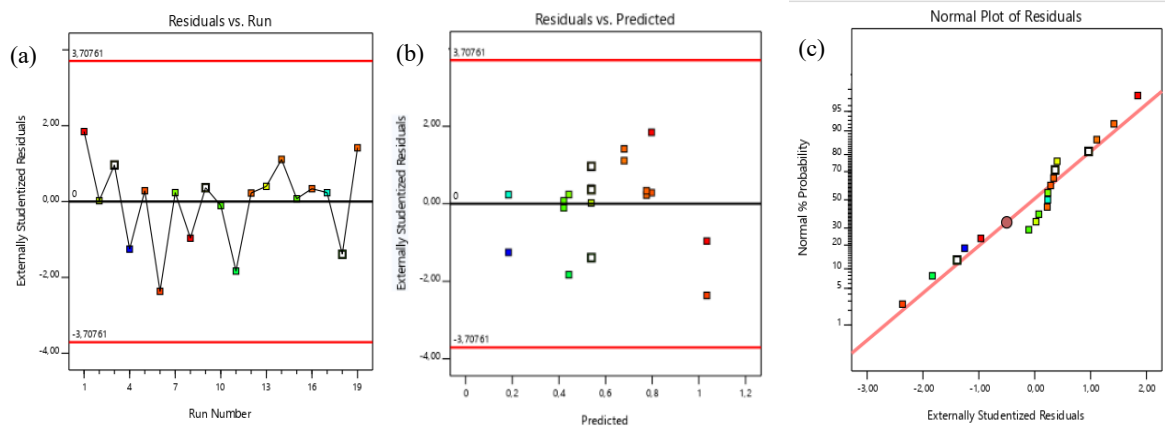


Figure 4-17: Residual Plots for Factorial Design

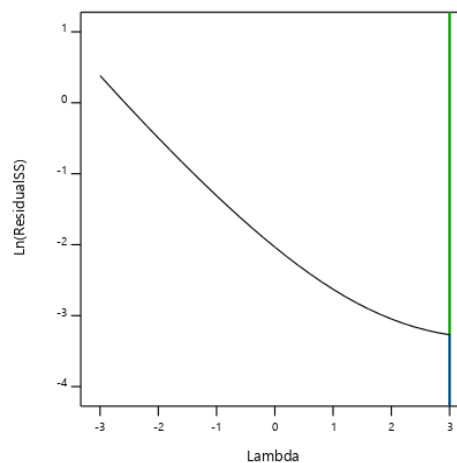


Figure 4-18: Box-Cox Plot for Power Transformations

4.5.3 Pareto plot

The Pareto plot is plotted to assess which experimental factor has the largest to the slightest effect. The plot is represented in Figure 4-17. T-statistics are calculated to test the null hypothesis that the effect is 0 and are termed standardised effects.

$$t_{\beta} = \frac{\beta - \beta_0}{SE(\beta)} \quad (4 - e)$$

Two reference lines are included, which indicate which factor effects are statically significant. The higher limit is based on Bonferroni/ family-wise corrected t-critical value, while the lower limit is based on standard t-critical for individual effects tests. Factor effects emerging above the Bonferroni limit are significant, while factors below this threshold are insignificant. Factor effects that lie between these limits may be significant. The Pareto plot indicates that the most influential factor is time followed by temperature then concentration. Furthermore, the interaction between time and concentration and the three-way interaction is significant, as shown in the analysis of variance.

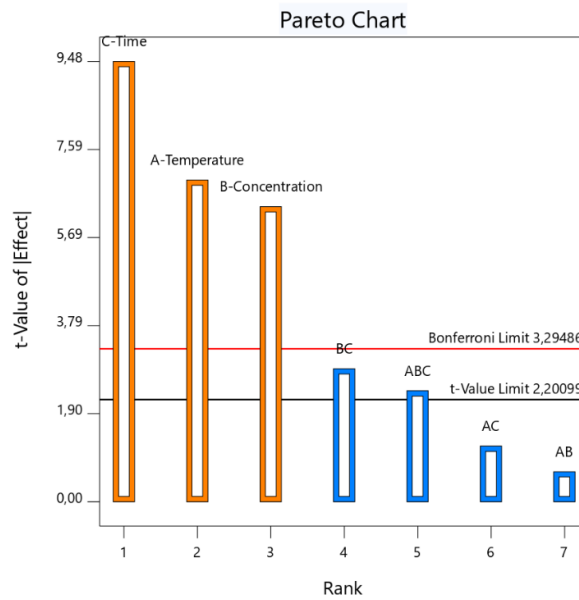


Figure 4-19: Pareto Plot

This observation is supported by the interaction plots denoted in Figure 4-18.

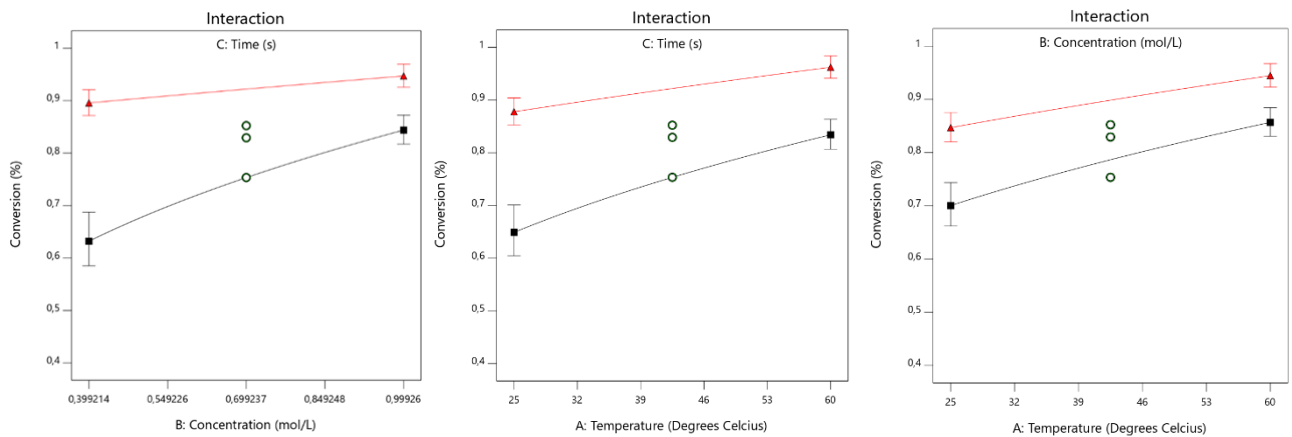


Figure 4-20: Interaction Plots

Interaction plots are graphical tools that help assess interactions in a factorial design. This plot displays one factor's levels against a separate line for each level of another factor. Thus, if lines are parallel, no interaction occurs, while nonparallel lines signify an interaction between the factors.

4.6 Summary of results

The xylitol results in both the factorial design and kinetic study proved to be inconclusive. The amount of xylitol detected far exceeded the stoichiometric limit and displayed erratic behaviour. Therefore, the study then shifted its focus on using xylose to determine the reaction kinetics and how reaction parameters affect the xylose conversion. The experimental results have been outlined in the above chapter. Statistical analysis of the results showed that the error in the measurements was statistically insignificant and can be further analysed. Nonlinear regression results were 1.25 and 1.37 in batch 1 and 2; this implies that the reaction rate follows first-order kinetics. Rate constants showed that the reaction speed increased with higher temperatures. Additionally, the activation energy and frequency factor were 31.74kJ/mol and 19146.72, respectively.

Furthermore, the factorial design showed that the most significant xylose conversion achieved was 98.86%, and the lowest conversion attained was 47.79%. According to the Pareto plot, the most significant factor was time, followed by temperature and then concentration. Furthermore, interactions between time and concentration and the three-way interaction were significant. This data allowed a model to be established to describe xylose conversion over a given range of set factors. The following chapter will build on the presented results and discuss their significance.

4.7 References

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Chapter 5: Discussion

5.1 Introduction

The purpose of this kinetic study was to establish the reaction order and reaction rate constant for the reaction between xylose and sodium borohydride. In addition, this investigation is done to assess the feasibility of alternate xylitol production methods. This chapter includes a discussion of significant findings related to the literature on the reduction reaction of aldehydes with sodium borohydride, varying structures of xylose, and its implications on the established mechanism. Also included is the factorial design, which provides an empirical model for the conversion over specific ranges for temperature, time and concentration. Finally, this chapter concludes with a discussion of the study's limitations and areas for future research.

The experimental data outlined in Chapter 3 show that the reaction order follows a pseudo-first-order rate law with rate constants of 3.43×10^{-2} 1/s and 9.76×10^{-3} 1/s at temperatures of 25 °C and 60 °C, respectively. Furthermore, the factorial study indicated that temperature is the most prevalent factor tested in improving conversion, followed by time and concentration. Interaction factors between time and concentration and the three-way interaction are significant. Xylose conversions obtained are comparable to those obtained in hydrogenation techniques. However, xylitol concentration has shown to be erratic and exceeds stoichiometric laws and possible reasoning for this behaviour will be highlighted in this chapter.

Despite erroneous xylitol concentration data, replicate xylose concentration showed reproducible results. Thus, reaction kinetics were established using xylose

concentration data. These results conform to the literature on reaction kinetics on sodium borohydride reductions and the associated mechanism.

5.2 Reaction kinetics

Minitab's estimates show that the reaction order is 1.37 and 1.25 for batches 1 and 2, respectively. However, a comparison between p values and significance level in the ANOVA analysis is required to ascertain whether the model appropriately postulates the relationship between the response and the predictors. This test assesses the null hypothesis, indicating no association between the term and the response. A significance level of 0.05 is chosen, indicating a 5% risk of concluding that a false association exists. Therefore, the p-value is greater than the significance level; hence the test does not detect a significant lack of fit.

The half-life of this reaction is 54 seconds and 9 seconds at 25 °C and 60 °C, respectively. The reaction reaches completion after 225 seconds at 60 °C and reaches a maximum conversion of 98.89% at 25 °C (Table 5-1). These results are similar to early experimental claims made by Abdek-Akher, Hamilton and Smith in 1951. This early study looked at the reduction of various sugars to their corresponding alcohols using sodium borohydride. The experimental method used was similar to the one conducted in this study. A solution of sugar (1.0 g) in water (20ml) was added to a diluted sodium borohydride at room temperature (20-25°C). The reaction mixture was then quenched using acetic acid to destroy excess sodium borohydride. Their study reports that the total conversion of xylose after 5 minutes with a yield of 80% was obtained for the acetylated product, xylitol pentaacetate (Abdek-Akher, Hamilton and Smith, 1951).

Table 5-1: Summary of Reaction Progress

Reaction Completion	Batch 1 (25 C)	Batch 2 (60 C)
25%	13s	4
50%	54s	9
75%	115s	12
100%	-	225

The residual plots are assessed to determine whether the nonlinear regression analysis provides reasonable estimates. The residuals vs fits plot (Section 4.2.3, Figure 4-10) are randomly scattered around the x-axis on both figures and form a rough horizontal band. This pattern supports the assumption that the relationship is linear and the variance of error terms are equal. Furthermore, no one residual deviates significantly from the residuals' basic pattern, which suggests no outliers.

The residuals vs order plot (Section 4.2.3, Figure 4-11) shows no trends or patterns when displayed in time order. In this case, time predictors should be added to the model. Furthermore, residuals exhibiting normal random noise around the residual x-axis suggest no serial correlation. The residual versus order plot illustrated exhibits no significant patterns, and therefore, we can conclude that residuals are not correlated (Martin, Adana and Garcia Asuero, 2017).

Therefore, the following estimates for kinetic parameters are reasonable and indicate the rate law can be represented as follows:

$$\frac{dC_{xyl}}{dt} = -k'C_{xylose}^{1.31 \pm 0.08}; k'(25^{\circ}\text{C}) 3.4 \times 10^{-2} \pm 5.67 \times 10^{-3} (5 - a)$$

$$; k'(60^{\circ}\text{C}) = 9.76 \times 10^{-3} \pm 4.20 \times 10^{-3} \quad (5 - b)$$

This claim supports the assumption that the first mechanistic step is rate-controlling and controls the overall reaction rate. However, it cannot prove the assumption only support it, as this study only provides a pseudo-order rate law. Despite the lack of

conflicting studies on the mechanism of reducing carbonyl compounds by sodium borohydride, all kinetic studies show an overall second order, first order in both the aldehyde/ketone and borohydride (Yamamoto, 2003). This theory is reinforced by kinetic studies conducted on other aldehydes reduced by sodium borohydride. Vanillin, a sweet-smelling aromatic compound, is reduced using sodium borohydride to produce vanillyl alcohol used widely as a flavouring ingredient. This reaction is commonly used in chemistry lab practicals, and the reaction rate is known to follow first order in sodium borohydride and the carbonyl compound (Lecher, 2007). Abe and Yamataka reduced benzaldehydes with borane (BH_3) in tetrahydrofuran (Abe and Yamataka, 2012). The study showed that the reaction order with respect to borane was 1.6 and that the reaction proceeds through a rate-determining hydride-transfer transition state with one molecule of borane acting as a reducing agent while the other serves as a catalyst. In 2006, Yadav and Lande investigated the reduction of citronellal to citronellol. Citronellal was reduced using sodium borohydride in liquid-liquid phase transfer catalysis using tetrabutylammonium bromide (TBAB), tetrapropylammonium bromide (TPAB), tetraethyl ammonium bromide (TEAB) and tetrabutylammonium hydrogen sulfate (TBAHS). The order was shown to range between 1.46 and 1.01 under different experimental conditions (Yadav and Lande, 2006).

Additionally, the estimates for the reaction rate constants are $3.4 \times 10^{-2} \text{ 1/s}$ and $9.76 \times 10^{-3} \text{ 1/s}$ in batches 1 and 2. These results indicate that higher temperatures increase the reaction rate, which supports collision theory. This data also suggests that xylose is a more potent reducing species at higher temperatures due to the shift

towards the acyclic structure of xylose at increased temperatures (Lepilova, Aleeva and Koksharov, 2012).

The Arrhenius equation determined the activation energy (E_a) and pre-exponential factor (A). The activation energy and frequency factors were determined at 25°C, and 60°C are 31.74 kJ/mol and 19146.72 kJ/mol. Expected E_a of bimolecular reactions lie typically in the order of 300kJ/mol; therefore, anything above this value would cause concern (Seoud, Baader and Bastos, 2016).

Table 5-2: Order of Magnitude of Kinetic Parameters (First Order Homogenous)

Reaction type	Activation energy, E_a (kJ/mol)
Slow $<10^{-3}$ to 10^{-2}	High >130
Moderate 10^{-2} to 10	Moderate 20-130
Fast >10	Low <20

(Marin and Yablonsky, 2011)

Furthermore, according to classifications in Table 5-2, this reaction can be categorised as moderate in reaction speed.

5.3 Factorial discussion

The 2^3 factorial design was conducted to provide an empirical model to predict xylose conversion over specific ranges for reaction temperature, time, and concentration. The lack of fit results for the model showed no error in the model. The R^2 is higher than 0.8, which is considered an acceptable fit for a model. The difference between the 'Adj R-squared' and 'Pred R-squared' is <0.2 ; this agrees with a general rule that a difference larger than 0.2 indicates issues with the data model. As the number of coefficients in the model approach, the number of design points, the predictions' ability tends to become unstable (Frost, 2017).

Initially, the normal probability plot showed an S-shaped curve, and the plot displayed a megaphone pattern. These trends suggest a case of expanding variance, and a transform is required. Referring to the Box-Cox plot, the optimal lambda (λ) value for the transformation was 3. Updated residual plots are shown in Figure 4-3, which shows a random distribution of the residuals, indicating constant variance and no signs of lurking variables that influenced the experiment's response.

The following table summarises the critical points of the experiment. The critical values for the factorial design show that increasing all factors to their respective maximum provides the highest conversion as expected according to the structure of xylose and collision theory (Lepilova, Aleeva and Koksharov, 2012; Fogler, 2016).

Table 5-3: Critical Points in Factorial Design

Critical Points	Test	Conversion	Conditions		
			Temperature (°C)	Concentration (mol/L)	Time (s)
Highest Conversion	6	0.99	60	1	300
Lowest Conversion	4	0.48	25	0.4	50
Centre point conversion range	9;12;19	0.82-0.89	60	1	300

5.3.1 Comparison with alternative xylitol production techniques

A comparison of the available literature shows that reported conversion performance is similar to those achieved by top-performing hydrogenation techniques (Table 5-4). However, sodium borohydride reduction can achieve this conversion at temperatures 60°C - 90°C below current commercial techniques

without a catalyst present. Additionally, this relatively simple technique produces similar conversions in a much shorter period.

Table 5-4: Summary of Top Performing Hydrogenation Techniques Reported

Catalyst	T (°C)	P(H ₂) (MPa)	Conv. (%)	Xylitol Yield
Ru/TiO₂	120	2.0	100	98
Ru/C	100-110	4.0-6.0	99.7	98.1
Raney Ni	120	5.5	96.6	93.7
Ru/NiO-TiO	120	5.5	99.9	99.7
Co/SiO₂	150	5	100	98

A recent study by Audema *et al.* in 2020 reported a 100% conversion of xylose with a 98% yield using hydrogenation techniques with a silica-supported monometallic cobalt Co/SiO₂ catalyst. The reaction period was 4 hours at a temperature of 150 °C with hydrogen pressure at 5.0 MPa (Audemar *et al.*, 2020).

Sodium borohydride reduction can achieve this conversion at temperatures 60 °C-90 °C below current commercial techniques without a catalyst. Additionally, this conversion is accomplished in a much shorter period. The advantage of hydrogenation is that catalysts can last for many cycles showing little degradation, whereas a significant amount of sodium borohydride is required to reduce xylose on a commercial scale. However, a techno-economic analysis is required to determine whether the cost of sodium borohydride would be cheaper despite lower operating conditions and no catalyst. Further optimisation techniques can improve this conversion, and therefore, this technique shows potential as an alternative process for producing xylitol.

Other viable xylitol-producing methods have been studied extensively, such as the biotechnological conversion of xylose to xylitol. These methods provide an

environmentally friendly and low-cost route to produce xylitol from hemicellulosic hydrolysates. Yeasts and filamentous fungi reduce xylose in a single step by a xylose reductase (XR) enzyme to produce xylitol and can be based on sugar mixtures such as lignocellulosic hydrolysates to save on energy and substrate purification costs (Dasgupta *et al.*, 2017). However, current literature shows that this technique is still not commercially competitive due to process bottlenecks; only a selected number of microorganisms can significantly reduce xylose, hindering optimisation space significantly (Buijs, Siewers and Nielsen, 2013). Additionally, glucose present in many microorganisms represses xylose metabolism through carbon catabolite repression (Park, Vinuselvi and Lee, 2012). Consequently, this leads to poor xylose conversion efficiency coupled with high reaction times.

5.4 Xylitol concentrations

Xylitol concentration data showed erratic behaviour and provided little information regarding the reaction kinetics. The first replicate of the experiment conducted at 25 °C is the only trial that provides meaningful results (Figure 5-1). This trial achieved a maximum conversion of 84.23 % and displayed a steady increase of xylitol that parallels xylose degradation. However, replicate data showed erratic behaviour and exceeded the stoichiometric ratio of 1:1 between xylose and xylitol, thus disobeying the conservation of mass. This erratic behaviour can be explained by the reaction mechanism where the alkoxide intermediate can act as a reducing agent or be reduced themselves, thus making it difficult to monitor the rate law (Carey and Sundberg, 2007). However, an apparent concern is the amount of xylitol produced. Specific runs in both experiments show that the xylitol yield is roughly three times the initial xylose concentration. These results question the reaction

mechanism's understanding; however, replicate results do not show the results to be reproducible and thus cannot be used to make a convincing case for a reversible reaction occurring. This issue in these results assumes that there is some experimental error involved in this study.

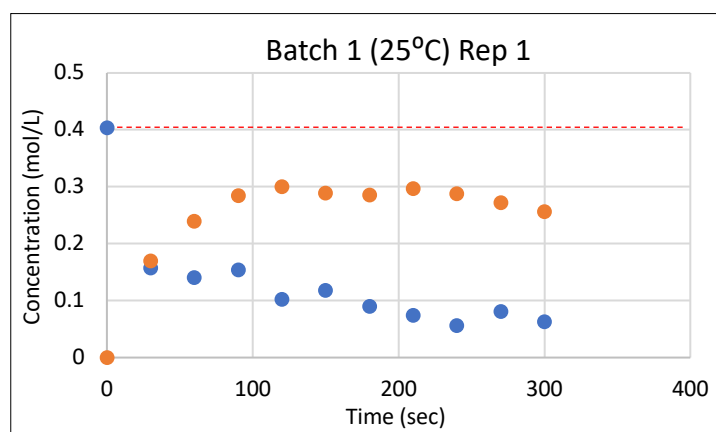


Figure 5-1: Concentration Data for Batch 1, Replicate 1

Samples were reanalysed to assess whether any issues existed in the HPAEC-PAD analysis. Results deviated slightly, indicating no error in the accuracy of this tool. Additionally, these results also showed little degradation in xylose and xylitol concentrations over three months. A possible reason for this occurring is that the HPAEC-PAD tool detects intermediates and quantifies them as xylitol in solution. However, xylose concentration data showed adequate error in replicate results to infer reasonable conclusions; therefore, a lack of experimental accuracy cannot be assumed in this case.

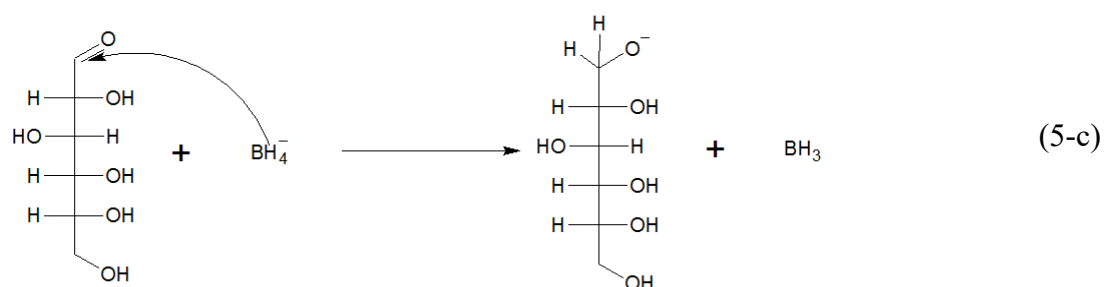
5.5 Analysis using pyrolysis GC-MS

The interpretation of the results in Py-GC-MS depends on the purpose of the analysis. Some results do not need interpretation beyond typical GC or GC/MS data analysis. Other results require specific interpretation to link the analysis results with

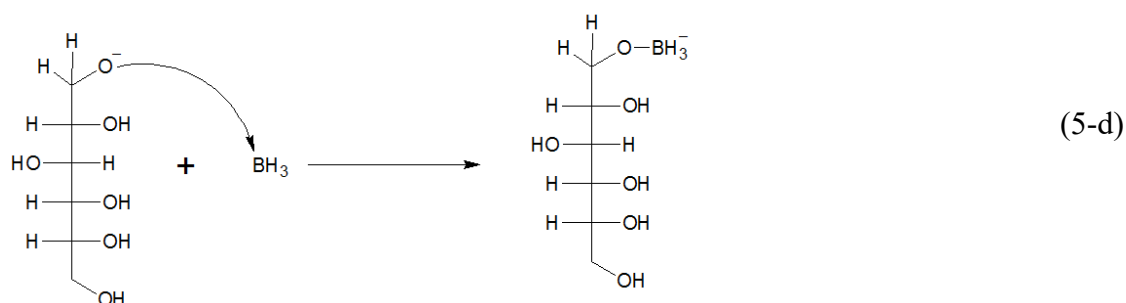
the initial material subject to pyrolysis. Because it requires further interpretation, it is not suitable in this case, especially when multiple aldehydes are present.

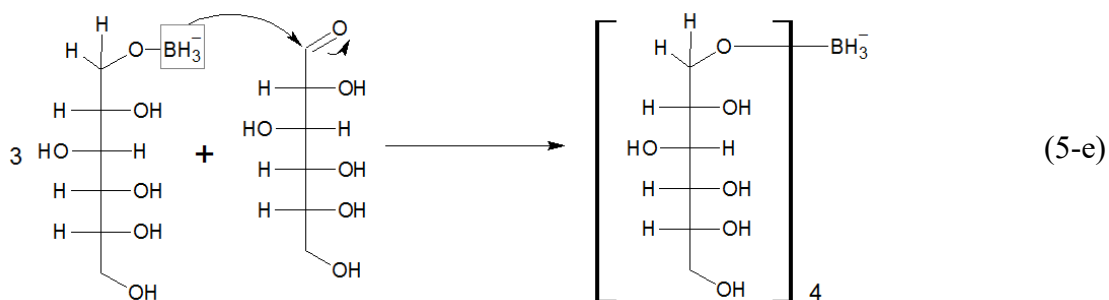
5.6 Mechanism

The mechanism shown below is theorised on the assumption that sodium borohydride follows a first-order rate law. This assumption is based on previous literature, indicating that sodium borohydride and the carbonyl compound follow first-order rate laws (Yamamoto, 2003).

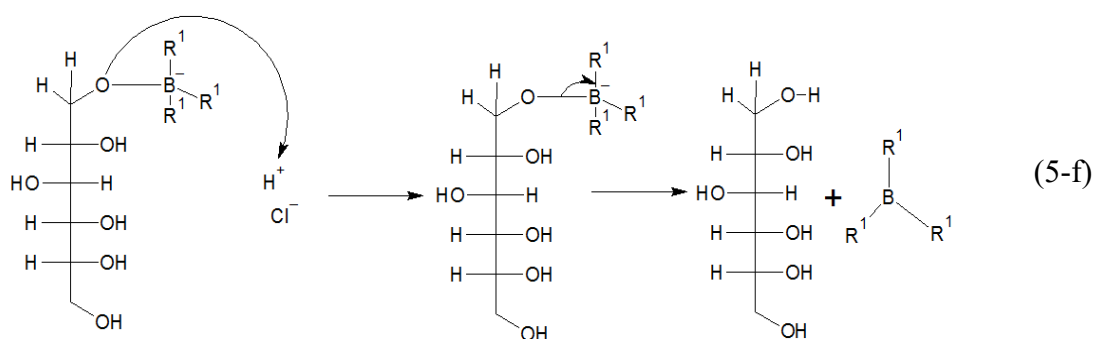


NaBH_4 reduction of aldehydes and ketones consists of two mechanistic steps: The above reaction equation depicts the nucleophilic attack on the carbonyl group by tetrahydroborate (BH_4^-), producing an alkoxide intermediate and borane. Following this step, two different reactions can occur.





This first approach (5-c) shows that the alkoxyborohydrides produced by the first addition of a hydride can also function as reducing agents, which reduce xylose. In the second approach, borane follows the first step and reduces subsequent xylose molecules.



The final step (5-f) involves the alkoxide anion protonated by H^+ from either the solvent or the acid added to give the final alcohol product.

5.7 Recommendation for future research

Since the discovery of sodium borohydride, the functionality of this compound has been expanded with chemical modifications, different solvents and novel techniques. These techniques have enhanced the reduction capabilities of sodium borohydride, and future studies can apply these methods to optimise xylose reduction.

5.7.1 Choice of solvent

Sodium borohydride can reduce most aldehydes; however, the rates of these reductions are determined mainly by the concentration of the solvent used and the reaction temperature. The solvent selection is based on the solubility and the rate of hydrolysis of sodium borohydride. (Section 2, Table 2-2). Suitable solvents for NaBH_4 are acidic alcohols. However, primary alcohols with low molecular weights (methanol, ethanol and ethylene glycol) are unsuitable if no base is present due to solvolysis discussed in Section 2.6.3. Secondary and tertiary alcohols provide stability for NaBH_4 , which is suitable even at elevated temperatures. Aprotic polar solvents can also be used, such as glymes, DMAC, DMSO, sulfolane, HMPA, NMP, pyridine, and dioxane, because sodium borohydride is stable indefinitely. Caution should be taken when conducting studies using dimethylformamide (DMF) as reports have shown violent exothermic reactions occurring under certain conditions and should be substituted with dimethylacetamide. Furthermore, a mixture of water and tetrahydrofuran (THF), a heterocyclic solvent with a low boiling point, accelerates the reduction of carbonyl compounds in sodium borohydride with simple solvent separation (Zeynizadeh and Behyar, 2005).

The use of ionic liquids has been explored as a potential replacement for organic solvents. The benefit ionic liquids provide is that they can be recycled and solvate numerous organic and inorganic compounds in a non-volatile manner. Howard and Ryan used this method by reducing a variety of aldehydes and ketones with sodium borohydride in liquid [bmim]PF₆. The results showed a reduction of benzaldehyde accomplished in one hour with a 90% yield. Furthermore, this method allows the alcohol produced to be distilled directly from the ionic liquid, thus making classical

organic solvents irrelevant (Howarth and Ryan, 2006). Recently Gayathri *et al.*, conducted reductions of various aldehydes using sodium borohydride with two ionic liquids, 1-hexyl-1,2,4-triazolium methanesulfonate and 1-hexyl-1,2,4-triazolium trifluoroacetate. The maximum yield achieved was 80% for benzaldehyde. Reaction ranged between 5 to 14 hours; however, reductions without ionic liquids required significantly higher reaction times. Therefore, this work showed that adding ionic liquids to reduce aldehydes and ketones by NaBH₄ improves reduction capabilities (Gayathri *et al.*, 2020). Therefore, a factorial design of differing solvents should be conducted to understand how it can impact xylose reduction.

5.7.2 Catalysts and co-reagents

The use of transition-metal catalysts and co-reagents has been shown to enhance the reactivity of sodium borohydride and is applicable to enhance the reduction of xylose (Wade, 1983). Compared to homogenous systems, heterogeneous catalysts provide various benefits, such as practical recycling and recovery and enhanced stability. In 2015, Rayati, Bohloulbandi and Zakavi reduced aromatic and aliphatic aldehydes using NaBH₄ in the presence of oxovanadium(IV) Schiff base complex encapsulated in the nanopores of zeolite Y (VOL-Y). The reactions were completed within 2 min, and yields vary between 74-100%. Furthermore, reductions of 4-chlorobenzene showed total conversion; however, in the absence of VOL-Y, only achieved maximum yields of 25% (Rayati, Bohloulbandi and Zakavi, 2015).

Another study utilised ammonia borane (AB) as a co-reagent to enhance the reduction reaction between sodium borohydride and various carbonyl compounds.

Ammonia borane is an easily handled, non-toxic and eco-friendly reagent. The experiment was conducted in water and achieved quantitative conversions with yields ranging between 82-97% within 5-10 minutes. Therefore, the excellent improvement in reaction times and yields reported provides an appealing approach to reducing xylose.

Several reductions of aldehydes and ketones with sodium borohydride under solvent-free conditions have been conducted. This procedure provides a solvent-free reaction, which has advantages from an ecological point of view. A significant disadvantage of this method is the long reaction times which can last for up to 5 days (Toda, Kiyoshige and Yagi, 1989). However, this method has been substantially improved, as reported by Cho *et al.* Sodium borohydride is activated by solid acids such as boric acid, benzoic acid, and p-toluenesulfonic acid monohydrate to reduce aldehydes and ketones. This synthetic method offers high yields, selectivity and simplicity, with reaction times ranging between 5 to 10 minutes. Similar reductions were conducted in the absence of activators, and the reaction proceeded considerably slower (Cho *et al.*, 2006).

5.7.3 Alternative reduction methods

A method involving an electrolytic procedure was reported in 2014 to reduce certain aldehydes and ketones in the presence of sodium borohydride. This approach utilises a simple undivided cell to provide inexpensiveness and environmentally friendly reduction. Furthermore, in most cases, the yield results were better than those achieved in sodium borohydride reduction (Velázquez-Olvera *et al.*, 2014).

Microwave irradiation was introduced in an effort to provide green reaction techniques. This method utilises the transformation of electromagnetic energy into heat and drives chemical reactions rapidly and efficiently (Kidwai, 2001). Zeynizadeh and Setamdideh used this approach to reduce aldehydes and ketones by sodium borohydride in water. Reductions were completed within 15sec-5min, with yields ranging between 70-99%. Thus providing a green method that provides excellent yields in a rapid manner.

Lastly, due to time constraints, further testing needs to be conducted using waste samples obtained from SAPPI's pulping process to understand how introducing other reducible sugars in the solution will impact xylitol production.

5.8 Conclusions

Drawing from these discussions, the results build on existing evidence that sodium borohydride reductions follow first-order rate laws in reacting with the carbonyl compound. Despite the limitations of the analysis, a pseudo-order rate law was deduced based on xylose concentration data. This observation correlates with literature regarding reducing carbonyl compounds with metal hydrides and supports the theory that the first hydride transfer is the rate-determining step. Furthermore, the factorial design provided an empirical model to assess xylose conversion over chosen reaction parameters. A maximum conversion of 98.8% was achieved at conditions significantly milder compared to hydrogenation techniques. Thus, the results obtained provide a basis for further study and indicate that the reduction of xylose with sodium borohydride shows the potential to compete with hydrogenation techniques. Lastly, the study allows for several exciting techniques to be explored regarding the reduction of xylose.

5.9 References

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Chapter 6: Concluding remarks

6.1 Introduction

The research reported in this thesis has investigated the reduction of xylose using sodium borohydride. This investigation was pursued to assess whether this reduction technique can be implemented in a commercial setting. An experimental technique has been developed that uses HPAEC-PAD to measure concentration changes in both xylose and xylitol to gain kinetic information. Furthermore, a factorial design was implemented to understand how certain factors affect the conversion of xylose. This final chapter summarises the significant findings of this study and outlines the limitations of the work.

6.2 Overview of the study

Chapter 1 outlined the background to the research problem by stating the problem, specifying the research objectives, identifying the gap in the literature and laying out the research approach. The proposed methods and structure of the research was described.

Chapter 2 reviewed the research literature relating to the historical background of sodium borohydride reductions and xylitol production. The survey outlined the perception of the reduction mechanism and factors which affect reaction rate and NaBH_4 decomposition. The review aims to establish a theoretical understanding and a basis for developing the research instruments used.

Chapter 3 examined the rationale for the research design and methodology. Various analytical methods were outlined, and justifications for the research methodology

is provided. Furthermore, techniques of data collection and result interpretation are included.

Chapter 4 presented the results obtained from the experimental procedures. Furthermore, this chapter includes the statistical analyses and interpretation of the results.

Chapter 5 discusses the results and forms assertions based on a statistical analysis of previous literature on sodium borohydride reductions. Furthermore, the feasibility of this reduction method is assessed relative to other reduction techniques.

Chapter 6 summarises the research and the conclusions to be drawn and scrutinises the study's limitations.

6.3 Summary of significant findings

6.3.1 Kinetic study

Analysis of xylose concentration-time data showed that the reaction order with respect to xylose follows a first-order rate law. This finding supports the mechanistic theory that the first hydride transfer is the rate-determining step. Rate constants were reported to be 3.4×10^{-2} 1/s and 9.76×10^{-3} 1/s at 25 °C and 60 °C. Additionally, the activation energy and frequency factor were determined to be 31.74 kJ/mol and 19146.72 kJ/mol, respectively. According to these parameters, the reaction speed is classified as moderate.

6.3.2 Factorial design

The influence of specific parameters is studied: initial xylose concentration, temperature, and reaction time. The estimation and the comparison of the

parameter's effects are accomplished by using a two-level factorial design. This experimental design provides an empirical model for the determination of xylose concentration over these factors. The analysis of these reaction factors indicates that the most significant factor is time, followed by temperature, then concentration. Furthermore, interactions between time and concentration and the three-way interaction are significant. The maximum xylose conversion achieved was 98.89% at 60° C, which is comparable to xylose conversions achieved in hydrogenation techniques.

6.4 Limitations of research

Due to the lack of data that xylitol provides, the results cannot provide accurate reaction yields. Additionally, the study cannot prove any hypothesis for the extreme xylitol yields. However, the erratic behaviour can be explained by the reaction mechanism.

The kinetic study provides a pseudo-order rate law that establishes a first-order rate law for xylose. However, a pseudo-order rate law does not provide an overall rate law and does not provide information on the order of sodium borohydride. Thus, the mechanism provided is only assumed based on previous literature on borohydride reductions.

Lastly, the factorial design study provides a linear response for xylose conversion and therefore, optimal reaction conditions are not located within the design space. However, the empirical model does provide useful conversion information over the selected conditions.

Appendices

Appendix A: Solubility of NaBH₄ in various solvents

Solvent	Temperature (°C)	Solubility (g/100g solvent)
Water	0	25.0
	25	55.0
	60	88.5
Liquid Ammonia	25	104.0
Methylamine	-22.0	27.6
Ethylamine	17	20.9
N-Propylamine	28	9.6
Iso-Propylamine	28	6
Cyclohexylamine	28	1.8
Morpholine	25	1.4
	75	2.5
Aniline	75	0.6
Pyridine	25	3.1
	75	3.4
Monoethanolamine	25	7.7
Ethylenediamine	75	22.0
Methanol	20	16.4
Ethanol	20	4.0
Iso-Propanol	25	0.37
	60	0.88
Tert-Butanol	25	0.11
2-Ethylhexanol	25	0.01
Tetrahydrofurfuryl Alcohol	20	14.0
Ethylene glycol dimethyl ether	0	2.6
	20	0.8
Diethylene glycol dimethyl ether	0	1.7
	25	5.5
	45	8.0
	75	0.0
Triethylene glycol dimethyl ether	0	8.4
	25	8.7
	50	8.5
	100	6.7
Dimethylformamide	20	18.0
Dimethylacetamide	20	14.0
Dimethylsulfoxide	25	5.8
Acetonitrile	28	2.0
Tetrahydrofuran	20	0.1

Adapted from (Yamamoto, 2003)

Appendix B: Chemical and physical properties of xylitol

Formula	C ₅ H ₁₂ O ₅
Molecular Weight	152.15
Appearance	White, crystalline powder
Odour	None
Solubility at 20 °C	169g/100g H ₂ O
pH in water (1g/10 mL)	5-7
Melting point (°C)	93-94.5
Boiling Point (760 mmHg)	216 °C
Density (bulk density) (15°C)	1.50 g/L
Caloric value 4.06 cal/g	16.88 J/g
Moisture absorption (%)	
60% (R.H)	0.05
92% (R.H)	90
Density (SG) aqueous solution (20°C)	
10%	1.03
60%	1.23
Heat of Solution	Endothermic, 36.61 cal/g
Viscosity (CP) 20°C	
10%	1.23
40%	4.18
50%	8.04
60%	20.63
Relative sweetness	Equal to sucrose, greater than sorbitol and mannitol
Specific Rotation	Optically inactive

Adapted from (Hyvönen, Koivistoinen and Voirol, 1982)

Appendix 3-A: Experimental trials for factorial design

		Factor 1	Factor 2	Factor 3
Std	Run	A: Temperature	B: Concentration	C: Time
		Degrees Celsius	mol/L	s
1	4	25	0.4	50
2	18	25	0.4	50
3	7	60	0.4	50
4	11	60	0.4	50
10	2	25	0.4	300
9	14	25	0.4	300
11	1	60	0.4	300
2	5	60	0.4	300
17	9	42.5	0.7	175
19	12	42.5	0.7	175
20	19	42.5	0.7	175
5	10	25	1	50
6	16	25	1	50
8	15	60	1	50
7	20	60	1	50
13	13	25	1	300
14	17	25	1	300
16	6	60	1	300
15	8	60	1	300

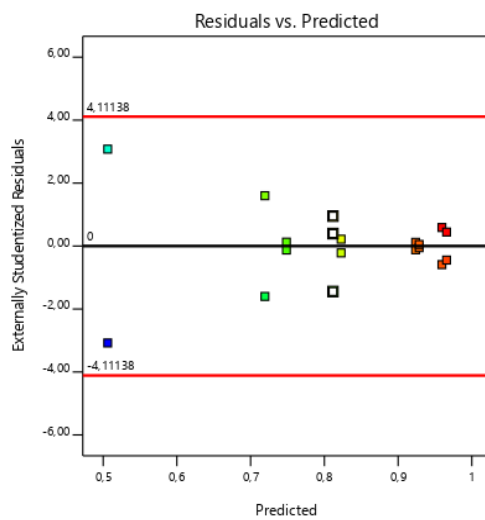
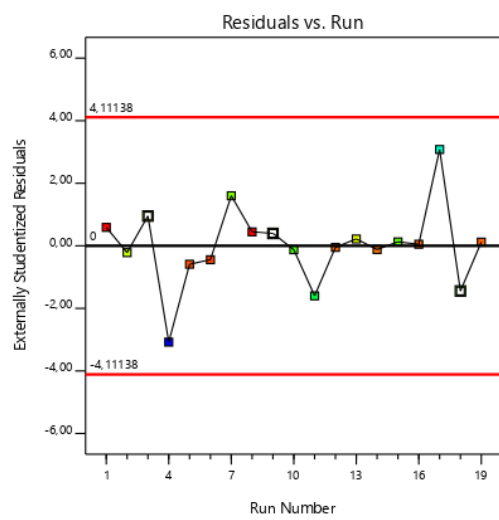
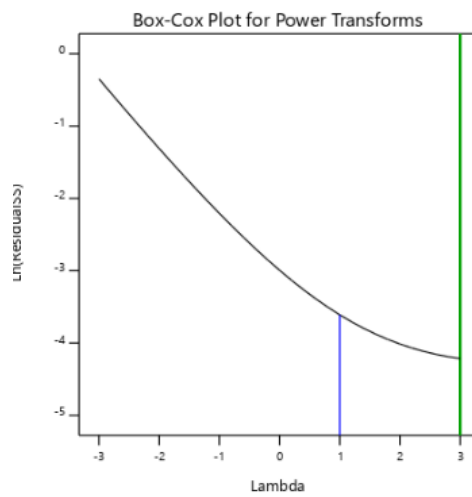
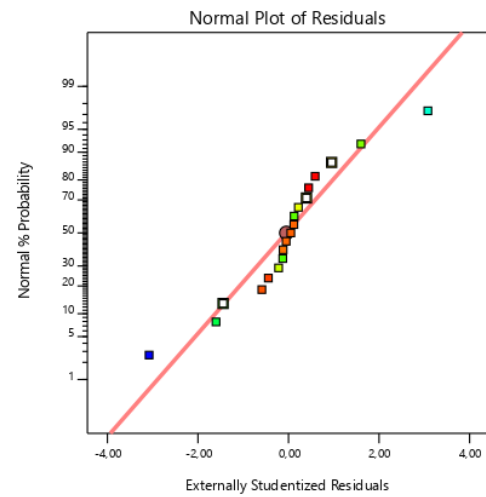
Appendix 3-B: Experimental setup



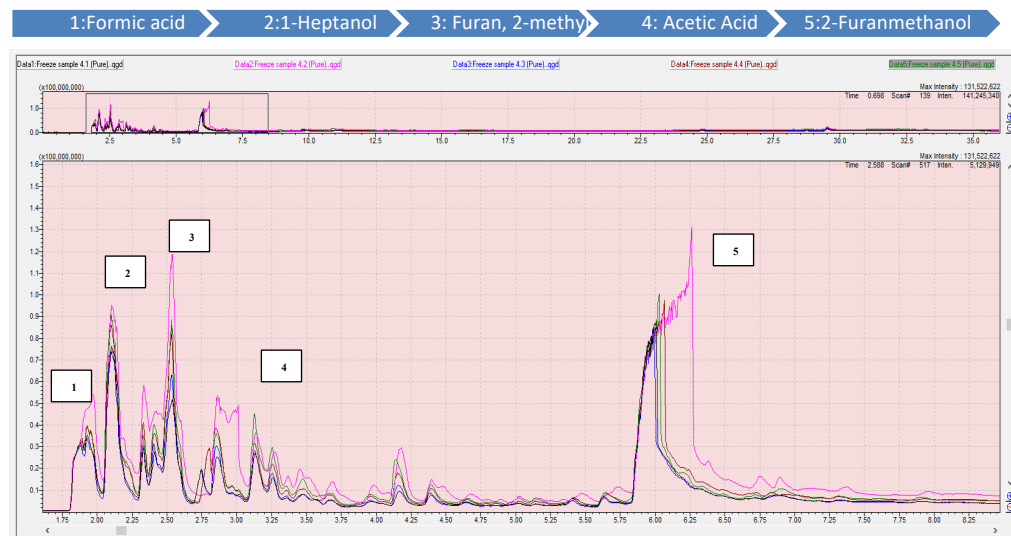
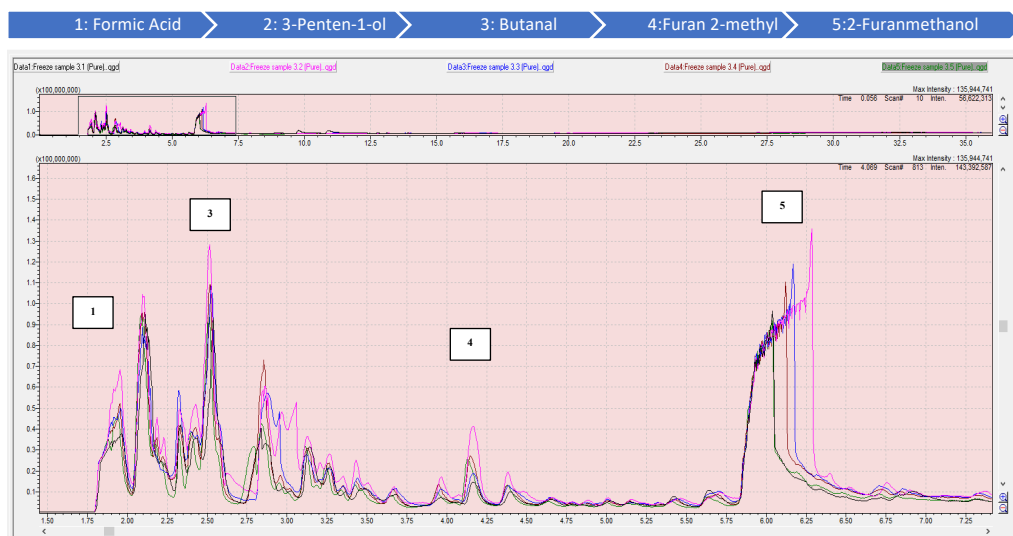
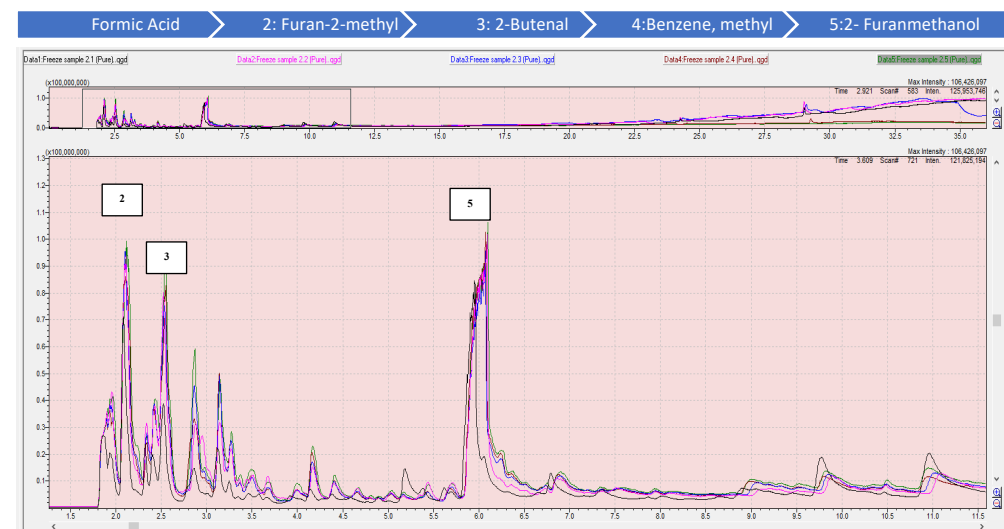
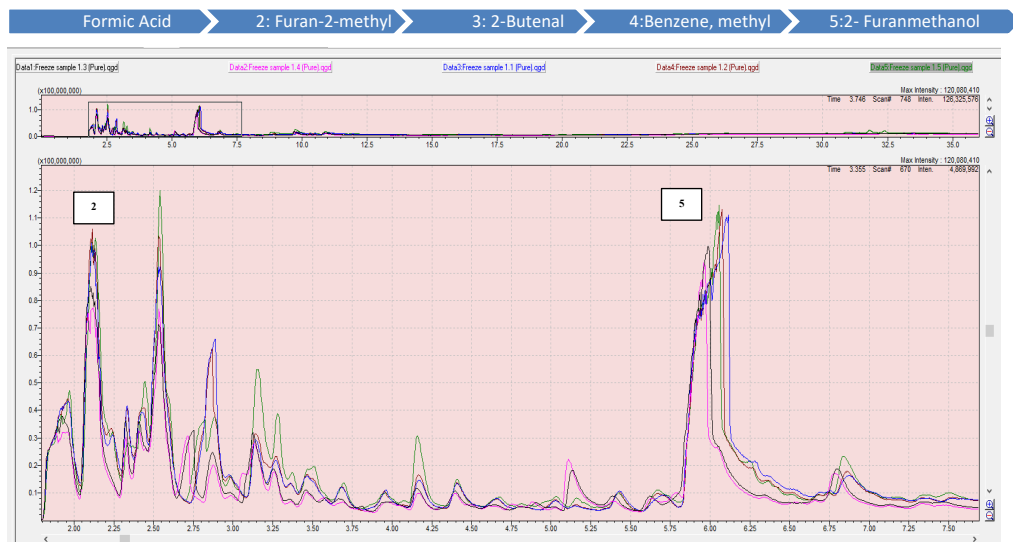
Appendix 4A: Standard deviation of xylose replicates

Time	Rep 1	Rep 2	Rep 3	Average	Standard Deviation
0	0.40	0.40	0.40	0.40	0.00
15	0.24	0.27	0.23	0.25	0.02
30	0.16	0.16	0.13	0.15	0.02
45	1.12	0.16	0.11	0.14	0.57
60	0.14	0.16	0.20	0.17	0.03
75	0.14	0.11	0.15	0.13	0.02
90	0.15	0.12	0.16	0.14	0.02
105	0.14	0.14	0.17	0.16	0.02
120	0.10	0.12	0.16	0.13	0.03
135	0.13	0.12	0.15	0.14	0.02
150	0.12	0.10	0.15	0.12	0.03
165	0.12	0.11	0.15	0.13	0.02
180	0.09	0.10	0.11	0.10	0.01
195	0.32	0.13	0.14	0.14	0.11
210	0.07	0.10	0.14	0.11	0.04
225	0.09	0.09	0.12	0.10	0.02
240	0.06	0.08	0.10	0.08	0.02
255	0.11	0.10	0.11	0.11	0.01
270	0.08	0.07	0.10	0.08	0.02
285	0.13	0.10	0.11	0.10	0.02
300	0.06	0.07	0.07	0.07	0.01

Appendix 4B: Initial residual plots for factorial design



Appendix 4C: Pyrograms



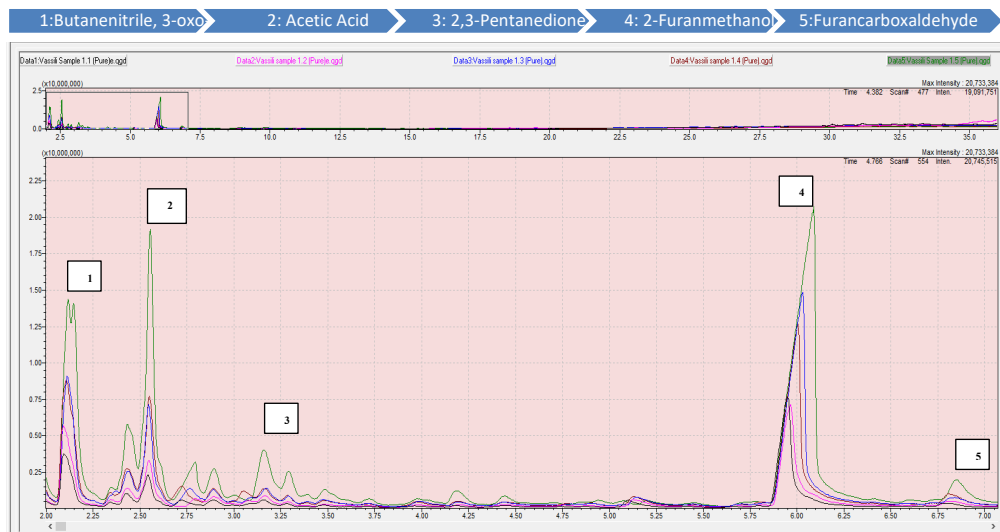


Figure 0-6:Pyrogram of Pure Oven-Dried Samples Batch 1.1-1.5

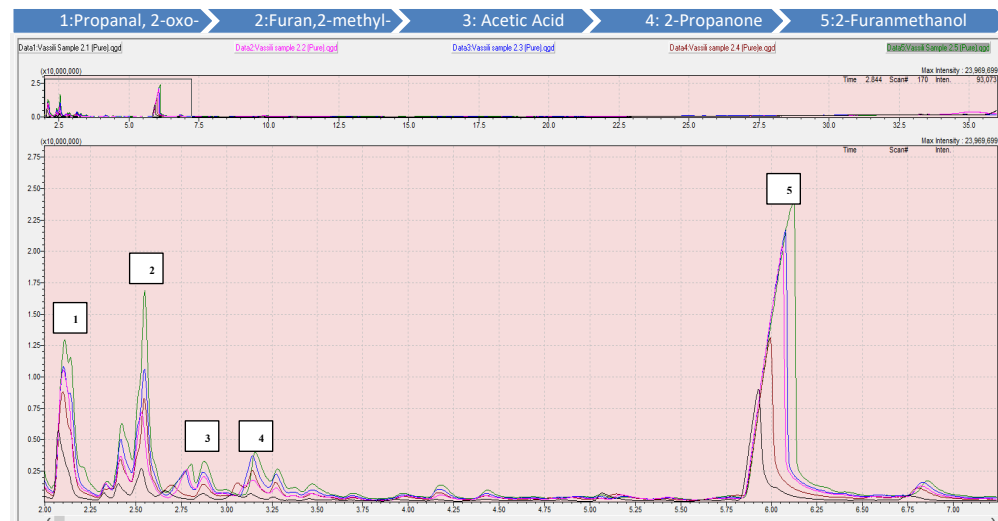


Figure 0-5: Pyrogram of Pure Oven-Dried Samples Batch 2.1-2.5

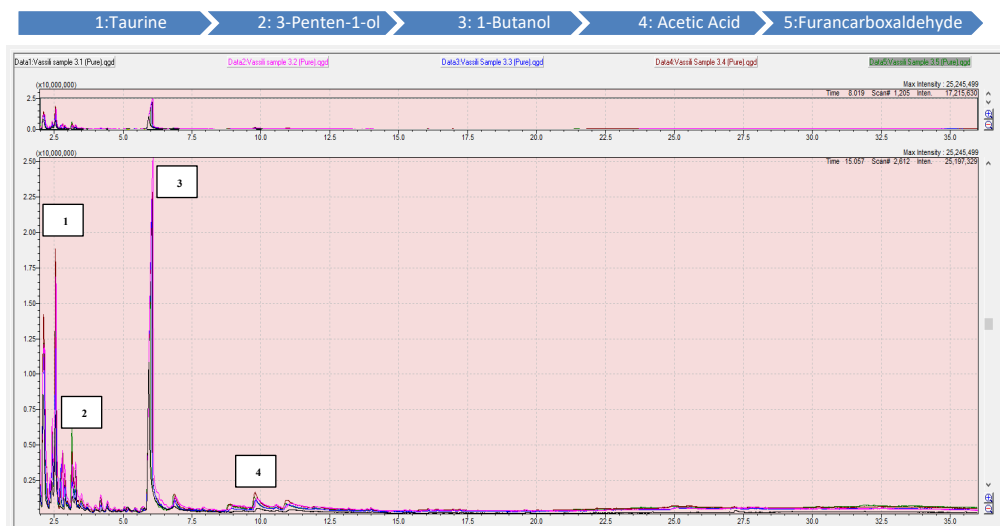


Figure 0-7: Pyrogram of Pure Oven-Dried Samples Batch 3.1-3.5

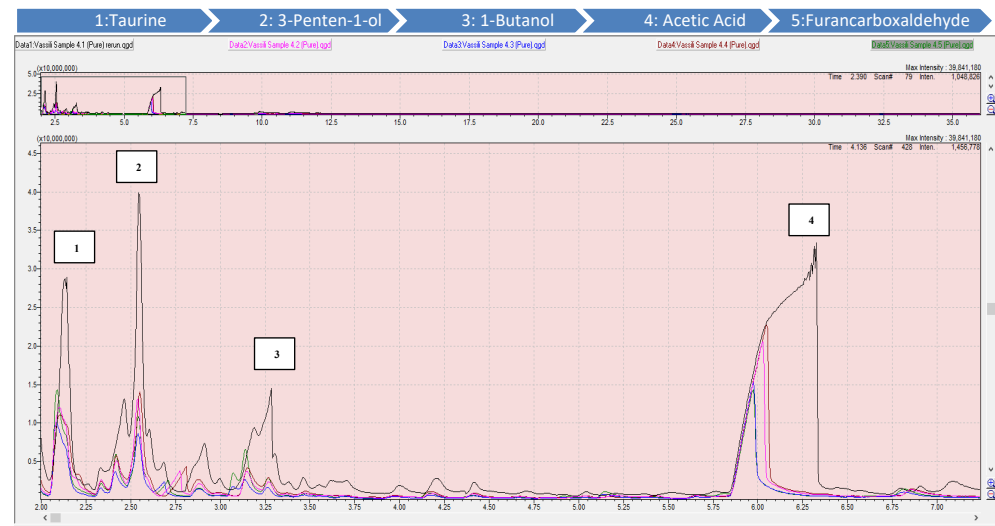
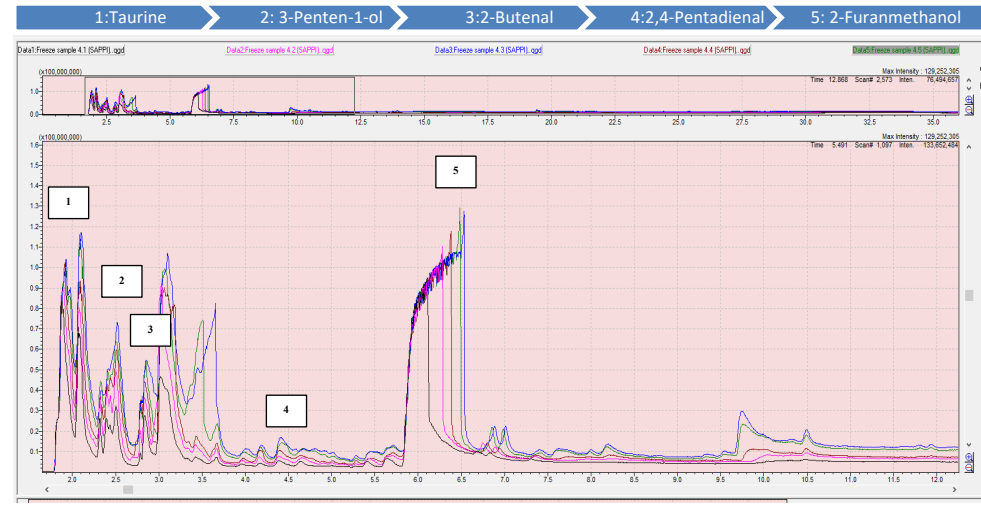
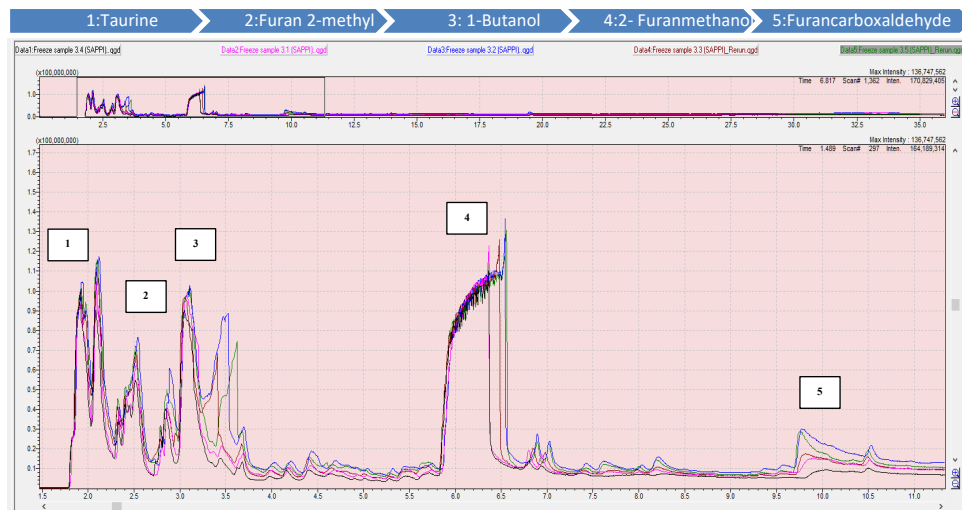
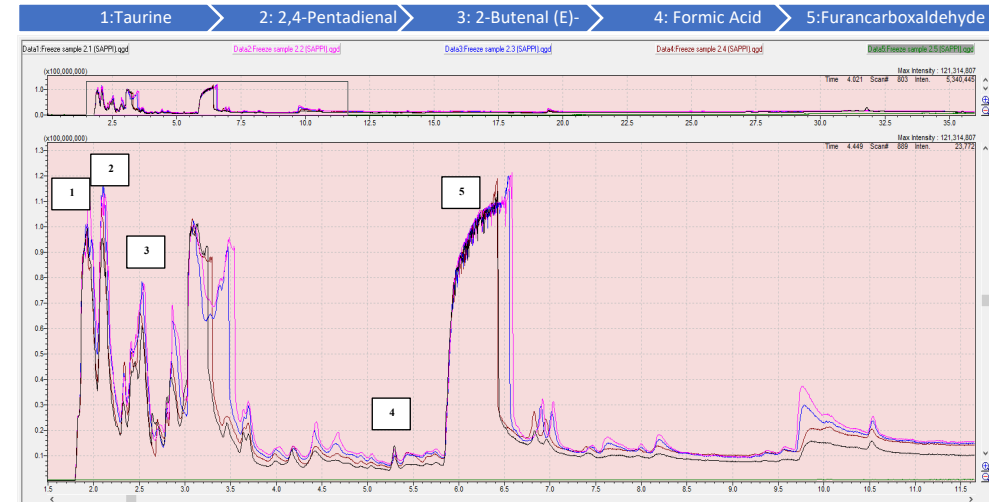
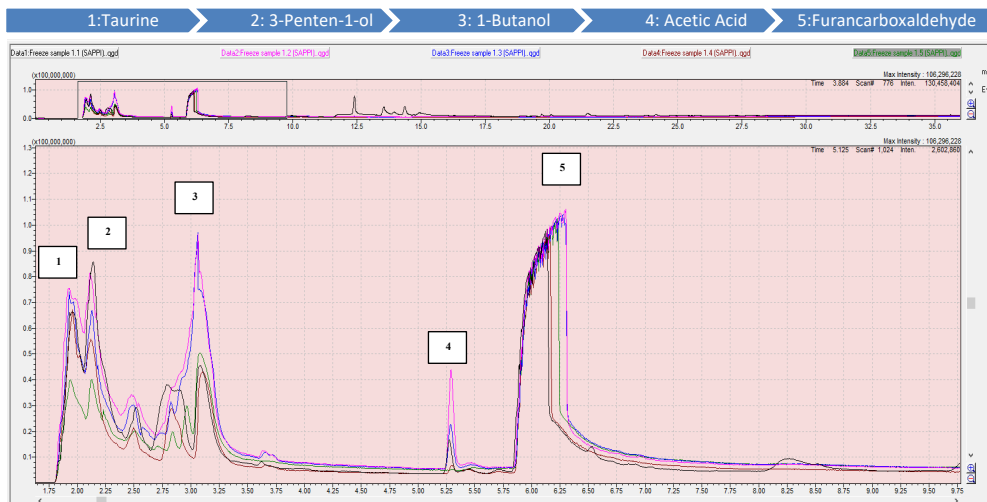


Figure 0-8:Pyrogram of Pure Oven-Dried Samples Batch 4.1-4.5



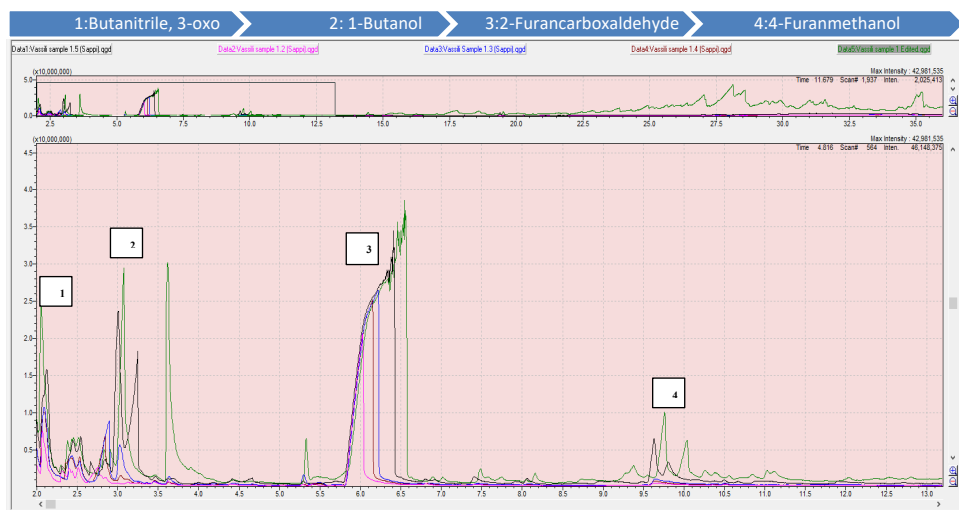


Figure 0-13: Pyrogram of SAPPI Oven-Dried Samples Batch 1.1-1.5

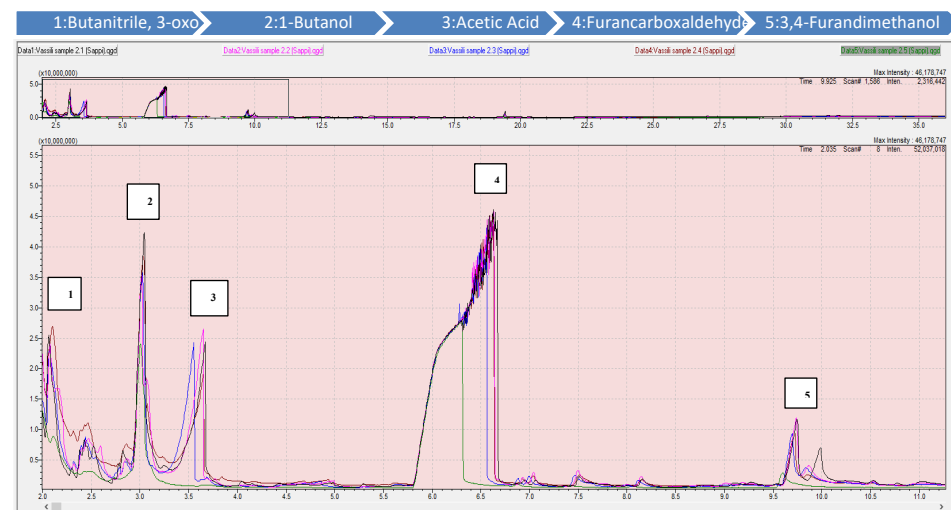


Figure 0-14: Pyrogram of SAPPI Oven-Dried Samples Batch 2.1-2.5

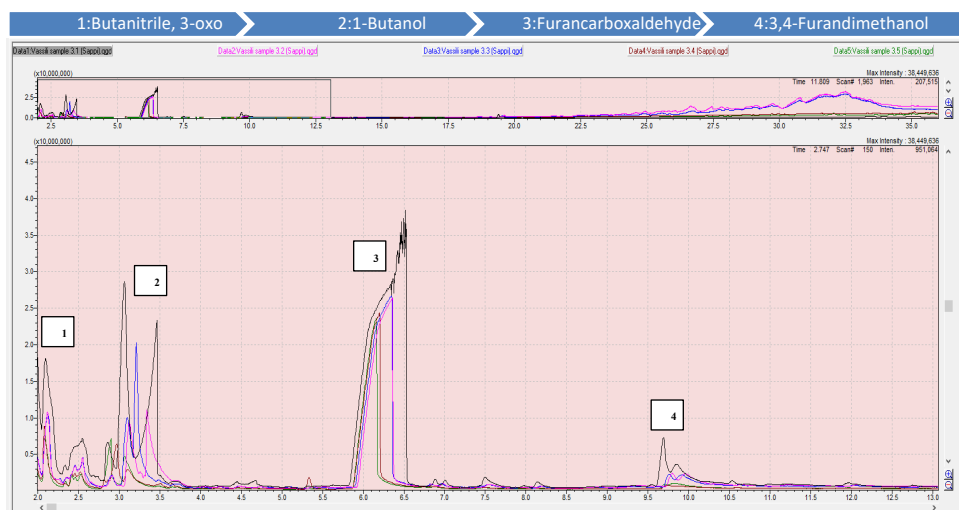


Figure 0-16: Pyrogram of SAPPI Oven-Dried Samples Batch 3.1-3.5

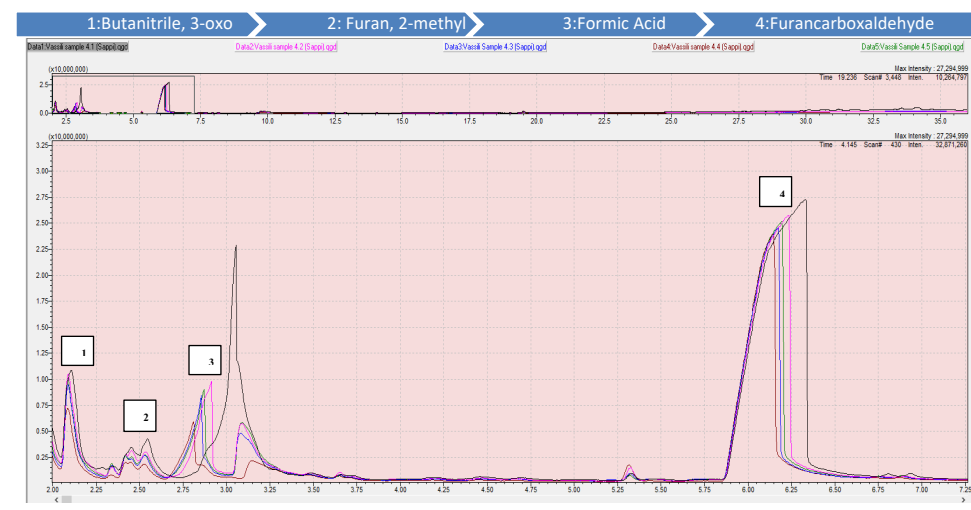


Figure 0-15: Pyrogram of SAPPI Oven-Dried Samples Batch 4.1-4.5