Theoretical and experimental studies on simultaneous-isomerization and reactive-extraction followed by back-extraction based process of biomass hydrolysate sugars

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A Dissertation

entitled

Theoretical and experimental studies on simultaneous-isomerization and reactive-extraction followed by back-extraction based process of biomass hydrolysate sugars

by

Kelly Renee Marbaugh

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Engineering

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The University of Toledo
December 2015
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An Abstract of

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The (bio)conversion of lignocellulosic biomass to biofuel and chemicals (such as ethanol and furans) requires both hexose and pentose sugars released to be fully utilized to make the process cost-effective. To better utilize these sugars, the aldose isomers of both hexose and pentose sugars can be isomerized into their more reactive ketose forms. However, the isomerization equilibrium of the both hexose and pentose sugars into the more reactive ketose isomers using the enzyme xylose isomerase (GXI) or other catalytic means predominantly favors the aldose sugars.

While there are multiple options for overcoming this unfavorable equilibrium, product removal strategies are widely used to shift the aldose/ketose equilibrium toward ketose. One such method, simultaneous-isomerization and reactive-extraction (SIRE), uses a two-phase system to improve the ketose yield during isomerization. In SIRE, the isomerization occurs in the aqueous phase and the sugar complexation occurs with a lipophilic boronic acid complexing agent (CA) confined to the organic phase. This approach enables reuse of the immobilized GXI and the complexing agent/organic phase.
It also enables the concentration of the sugars as they are stripped by back-extraction (BE) from the organic phase.

The goal of this dissertation research was to develop mathematical models to predict the results of SIRE-BE of biomass sugars glucose and xylose. These models were validated with experimental data and were used to predict the outcome of the SIRE-BE process under a variety of operating conditions. Experimental data was collected for sugar isomerization, reactive-extraction, and back-extraction to determine appropriate model parameters.

Kinetic parameters for the isomerization of aldose to ketose sugars were determined using experimental data fit to Michaelis-Menten reversible, enzyme-catalyzed reaction models. The theoretical model for the mixed sugar reactive-extraction process was derived as part of this dissertation. Equilibrium constants for the reactive-extraction of sugar isomers were determined from experimental data collected under several reactive-extraction conditions. The model was used to predict the composition of the resulting aqueous and organic phases at equilibrium and was implemented in MatLab. Parameters determined from the fits of isomerization and reactive-extraction models to experimental data were used in a comprehensive mathematical model that included both transient isomerization and pseudo-steady state reactive-extraction of aldose and ketose sugars to simulate SIRE.

By optimizing the results for SIRE-BE for the pentose sugar isomerization (xylose to xylulose), a xylulose-rich aqueous sugar stream can be produced and converted into a wide variety of platform chemicals. One example is the dehydration of xylulose into furfural; furfural can be used as a precursor for fuels, fuel additives, and biobased-
materials. Using existing experimental data for this dehydration reaction, a robust mathematical model of the reaction kinetics was developed to predict rates of xylulose consumption and furfural production under a range of temperature conditions.
For all of their love, encouragement and support throughout the years, I dedicate this dissertation to my family. I would not be where I am or who I am without you.

This dissertation is also dedicated in loving memory of my grandparents, Norbert and Pauline Kerner. I have felt your faith, love, strength and wisdom guiding me every step of the way.
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Chapter 1

Overview

1.1 Background

The petroleum refining industry greatly impacts the global economy. Crude oil is converted into a variety of products ranging from liquid transportation fuels, solvents and lubricants to feedstock chemicals for plastics. Fluctuating worldwide oil prices, coupled with increased demand and uncertainty in remaining oil reserves, has increased interest and attention for the development of alternatives to petroleum. Lignocellulosic biomass feedstock, which includes all plant and plant-derived material, is one such alternative renewable source to produce fuels and a variety of other chemicals. Biomass is a particularly attractive alternative because it is currently the only renewable sustainable energy source for liquid transportation fuel.

According to the National Renewable Energy Laboratory (NREL), a biorefinery is defined as integrated processes for conversion of biomass to fuels, power, and chemicals\(^1\). The biorefinery could take advantage of various components in biomass feedstocks to produce a full array of low-value, high-volume liquid transportation fuels such as bioethanol while generating power (heat and electricity) to meet energy needs; and high-value, low-volume bio-based products to increase profit. This concept is built
on two different “platforms” (Figure 1-1). The “sugar platform” is based on biochemical conversion processes and focuses on the fermentation of sugars extracted from biomass feedstocks. The “syngas platform” is based on thermochemical conversion processes and focuses on the gasification of biomass feedstocks and by-products from conversion processes. This dissertation focuses on producing highly-useful isomers of biomass-derived sugars which can be converted to products via thermo-catalytic routes. In particular, the optimization and modeling of sugar utilization, concentration and separation will be explored.

Figure 1-1: Modified from NREL process flow for Biorefinery concept.

1.1.1 Biomass: structure, deconstruction and sugar utilization

Wheat straw, corn stover and other agricultural residue along with forestry waste products from poplar are among the most important sources of lignocellulosic biomass. Lignocellulosic biomass is a complex structure composed of cellulose (40–50 wt %), hemicellulose (25–35 wt %), and lignin (15–20 wt %) (Figure 1-2). The exact
percentage of each biomass constituent varies depending both on the plant species and the plant tissue utilized. Cellulose is a linear polymer composed of glucose subunits linked by $\beta-(1\rightarrow4)$-glycosidic bonds. Linear cellulose chains usually take on a parallel arrangement with extensive hydrogen bonding between the chains, making enzymatic hydrolysis difficult\(^3\). Hemicellulose is a highly branched and complex heteropolymer that contains hexoses (D-glucose, D-galactose, and D-mannose), and pentoses (D-xylose and L-arabinose). Unlike cellulose, hemicellulose is easily hydrolyzed to its constituent monosaccharides\(^4\). Lignin is composed of variety of polyphenolic compounds. It also gives plant cell walls its rigid structure and protects cellulose and hemicellulose from enzymatic degradation. Cellulose and hemicellulose polysaccharides, which account for approximately 60 wt% of dry biomass, can be hydrolyzed to produce monosaccharides. Glucose and xylose, the major monomeric sugars, can be converted to a wide variety of downstream products.

Figure 1-2: Lignocellulosic biomass structure\(^5\).
1.1.2 Biomass processing

A general schematic for processing of biomass for the sugar platform is shown in Figure 1-3. After the biomass is harvested, it is mechanically broken into small, uniform pieces before moving on to the pretreatment step. Pretreatment is necessary before the biomass undergoes enzymatic hydrolysis. Without this pretreatment step, enzymatic hydrolysis yields fall well below the theoretical yield. The pretreatment process increases the surface area that is accessible to the cellulase enzymes, allowing for an enhanced enzymatic hydrolysis of the lignocellulosic portion of the biomass, which significantly increases yields of monosaccharides such as glucose and xylose. The resulting sugar solution, or hydrolysate, can be processed through a variety of methods to generate biobased products.

![Biomass processing to individual sugars.](image)

Figure 1-3: Biomass processing to individual sugars.

1.1.3 Furan production

Many products and chemical precursors that traditionally have been produced from petrochemical precursors can instead be produced from a biomass sugar platform. One such group of chemicals that shows immense promise to be produced from thermocatalytic treatment of biomass is furans\(^6,7\). Furans are the precursors for several polymers and hydrocarbon fuels. The dehydration of biomass sugars to furanic compounds such as furfural and 5-hydroxymethylfurfural (HMF) (Figure 1-4), is a first step in producing high-value products such as diesel fuel additives (by aldol condensation and aqueous phase dehydration-hydrogenation), industrial solvents (such as
tetrahydrofurfural alcohol and furfuryl alcohol) and various bio-derived polymers (by conversion of HMF into furan dicarboxylic acid, FDCA)\textsuperscript{8-10}.

Glucose and its isomer fructose can be converted to HMF through dehydration reactions, or/and subsequently converted to dimethylfurfural (DMF). The dehydration of xylose and xylulose leads to the formation of furfural, which is also an important platform chemical. Figure 1-5 shows the plethora of compounds and chemicals that can be produced from the dehydration of xylose into furfural.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{furan_production.png}
\caption{Schematic of furan production from biomass sugar platform. Cellulose and hemicelluloses can be converted to monomeric sugars. These sugars, glucose and xylose, can be dehydrated to furans, HMF and furfural, respectively. However, this process has poor yields due to side-product formation. By isomerizing the sugars to their ketose form (fructose and xylulose), the poor yield of furans can be overcome. Additionally, the dehydration of ketose sugars to furans can be achieved at lower reaction temperatures.}
\end{figure}
Figure 1-5: Furfural as a platform chemical. Furfural can be used to produce a wide variety of compounds such as ethyltetrahydrofurfuryl ether (ETE), methyltetrahydrofuran (MTHF), ethyl levulinate and tetrahydrofuran (THF). These chemicals can be used as biofuels or further converted to replace products derived from petroleum.

1.1.4 Challenges for biomass sugars utilization

While there is immense promise for the use of biomass sugars, there are three distinct limitations for downstream conversion to products. One such limitation is the relatively low concentrations of sugars resulting from pretreatment and enzymatic hydrolysis. Concentration of the sugars in the biomass hydrolysate can be achieved by boiling or vacuum evaporation, but both of these processes are energy-intensive, and not economically feasible on an industrial-scale. The second limitation is that contaminants formed by sugar degradation during production of the biomass hydrolysates (furfural, HMF, acetic acid, levulinic acid) and phenolic compounds from lignin degradation, tend to limit the efficiency of subsequent bioconversion of sugars. One common method to remove the contaminants from the biomass hydrolysates is adsorption by using activated carbon or ion-exchange processes. Although these conditioning steps are effective at removing the contaminants, they produce a great amount of solid waste that can be both
financially and environmentally taxing to safely dispose. Finally, ketose sugar utilization can be more effective and efficient than the corresponding aldose sugar. For example, baker’s yeast, which is typically used in the production of ethanol from biomass sugars, is unable to utilize xylose for ethanol production. Xylose accounts for about 30% of the total sugars found in biomass hydrolysate. To overcome this substantial challenge, genetically modified organisms (GMOs) have been produced to ferment xylose. The stability, product yield, environmental impact, and survival under conditions of industrial fermentation are unproven for these GMOs. However, isomerization of xylose to its ketose form, xylulose, allows native yeast to utilize this biomass sugar.

1.1.5 Improving sugar utilization by isomerization

While detoxification strategies can be employed to remove non-sugar compounds from hydrolysate, it may be more cost-effective and reliable to remove sugars from contaminants. Reactive-extraction schemes which transfer a desired compound between immiscible phases via the assistance of a chemical reaction, have the potential to achieve this goal. Borax, aryl boronic acids, and their derivatives can reversibly bind to sugars with pH-dependent affinities. By devising schemes that confine the resulting sugar complexes to an organic phase that is immiscible with water, it is possible to selectively extract sugars from a biomass hydrolysate at alkaline pH conditions. Unlike traditional extraction via selective partitioning, the reaction chemistries used to achieve confinement of the sugar complex to the organic phase allow the sugars to be concentrated upon their release into a separate, low pH aqueous back-extraction phase for subsequent reaction to products.
Reactive-extraction of sugars into an organic phase followed by back-extraction into a low pH aqueous phase has the potential to provide an economically-viable scheme to obtain clean, concentrated sugar solutions.

Biomass sugars in the hydrolysate can be isomerized to their ketose forms which may be advantageous in fuel and chemical production. Chemical structures of glucose and xylose and their ketose isomers are shown in Figure 1-6.

Figure 1-6: The structures of major biomass sugars and their ketose isomers.

Ketoses are typically more-readily converted to useful products than aldoses. Production of HMF from fructose is accomplished with higher yield and less by-product formation under less harsh conditions than are required for HMF production from glucose\(^9\).

Isomerization of aldoses to ketoses is catalyst-driven, with the reaction generally favoring the aldose form of the sugar. At equilibrium, only a small portion of the sugar is in the ketose form (40-50% fructose from glucose and 25-35% xylulose from xylose). Removing the ketose sugar as it is formed significantly enhances the overall yield of ketose sugar from this reaction\(^{15,16,23}\). A novel strategy for simultaneous-isomerization-and-reactive-extraction (SIRE), where the isomerization of aldose sugars and the reactive-extraction of ketoses occur simultaneously in the same vessel has been developed\(^{24-25}\). The reactive organic phase contains a complexing agent such as naphthalene-2-boronic acid (N2B) and a lipophilic amine salt, Aliquat® 336 (Q\(^+\) Cl\(^-\)). The
sugar contained in the organic layer can be back-extracted (BE) into an acidic aqueous solution that can be used for the production of bio-based products. SIRE-BE has already been tested with the pure sugar xylose and a nearly pure, concentrated xylulose solution with high yields was obtained\textsuperscript{37}. The organic phase composition was shown to significantly impact the product yield and purity, and as a result, experimental conditions needed for high conversion and recovery of other sugars, such as glucose converted to fructose, are yet to be determined.

1.1.6 SIRE-BE concept

A mechanistic framework for SIRE will facilitate the interpretation of the experimental results as well as guide experimental design. Figure 1-7A shows the overall reaction scheme proposed for simultaneous-isomerization-with-reactive-extraction\textsuperscript{37}. Isomerization of aldose sugar to the ketose isomer occurs in slightly alkaline aqueous media. The organic media contains both the lipophilic complexing agent (CA) and a lipophilic quaternary amine salt (such as Aliquat\textsuperscript{®} 336), shown as Q\textsuperscript{+}Cl\textsuperscript{−}, which aid the selective extraction of sugars from the aqueous media. As the ketose sugar is extracted into the organic media, the unfavorable isomerization reaction is shifted toward the ketose sugar. The pairing of isomerization with reactive-extraction effectively improves the overall isomerization due to product removal. The success of the SIRE technology hinges upon the ability to “selectively” extract the ketose sugar into the organic phase, while leaving behind the aldose sugar. Both sugar extraction and back-extraction will be described in Sections 1.1.7 and 1.1.8.

Figure 1-7B shows the scheme proposed for back-extraction (BE) of the extracted sugars from the organic media. When the sugar-laden organic media is contacted with an
acidic aqueous stripping solution, the sugar-CA complexes destabilize, releasing the sugar into the aqueous phase.

1.1.7 Extraction of sugars into organic phase

Lipophilic aryl boronic acids used as sugar complexing agents are not water soluble and do not partition into the aqueous phase, even after ionization to their conjugate base configuration at high pH. When an organic phase containing the weak acid CA is contacted with a high pH aqueous phase, the CA forms a conjugate base (CA\(^-\)) at the organic/aqueous interface as shown in Figure 1-7A. In the presence of sugar (S), the CA\(^-\) favors the more-stable ester form, CAS\(^-\), with sugar transferred from the aqueous phase. The CA\(^-\) and CAS\(^-\) anions are confined to the aqueous/organic interface due to their dual hydrophilic–lipophilic character. These negatively charged forms of CA are inherently dependent on the pH of the aqueous phase. If a substantial decrease in the pH of the aqueous phase occurs, these species of CA lose their charge. When the lipophilic amine salt Q\(^+\)Cl\(^-\) is added to the organic medium, the cation Q\(^+\) of the salt is able to form an ion pair with either CAS\(^-\) or CA\(^-\). These neutral organic-soluble ion pairs, (Q\(^+\)CAS\(^-\) or Q\(^+\)CA\(^-\)), are no longer restricted to the aqueous-organic interface and are able to dissolve freely in the organic phase. The anion of the salt, Cl\(^-\), passes into the aqueous phase to maintain charge neutrality in the organic phase.

The mechanism proposed in Figure 1-7A for ion-pair formation, along with maintaining an overall neutral charge, necessitates the movement of both H\(^+\) and Cl\(^-\) into the aqueous phase. When the phases are contacted, the pH of the aqueous phase decreases, requiring the addition of base, such as NaOH, to maintain the alkaline sugar
extraction and isomerization pH. The decrease in pH can be attributed to the ionization of the CA and the triagonal form, CAS, and release of $H^+$. Therefore, the moles of sugar

Figure 1-7: Hypothetical mechanism of simultaneous isomerization with reactive-extraction (SIRE). (A) During reactive-extraction, a complexing agent (CA), such as an aryl boronic acid, in contact with a high pH aqueous phase is readily converted to its conjugate base ($CA^-$) form. In the presence of sugar, the $CA^-$ forms a more-stable tetragonal ester with the sugar ($CAS^{-}$). The negatively charged ester is restricted to the interface of the aqueous and organic layers until ion-pair formation occurs with the cation ($Q^+$) of a lipophilic salt $Q^+Cl^-$ in the organic phase. In SIRE, xylose isomerase ($GXI$) catalyzed isomerization of aldose (A) to ketose (K) sugar occurs simultaneously with ketose-selective sugar extraction. (B) Stripping of sugars from the organic phase occurs by contacting the organic phase with an acidic aqueous phase (i.e. HCl solution). The $Q^+CAS^-$ loses an $OH^-$ group to the low pH aqueous phase and dissociates from the $Q^+$. In turn, the uncharged CAS dissociates; the CA remains in the organic phase while the sugar transfers to the aqueous phase. Chloride ion (from HCl) in the aqueous phase forms an ion-pair with $Q^+$ at the aqueous/organic interface, regenerating the quaternary ammonium salt and reducing the concentration of $Cl^-$ in the aqueous phase.
extracted to the organic phase are less than the moles of H$^+$ transferred to the aqueous phase. An equivalent amount of Cl$^-$ transfers to the aqueous phase due to the ion-pair formation of CA with the lipophilic salt cation, Q$^+$. 

1.1.8 Stripping of sugars from organic phase

The sugar extracted into the organic phase can be recovered by contacting the sugar-containing organic phase with a low pH HCl solution (Figure 1-7B). During sugar stripping, the Q$^+$CAS$^-$ ion pair is disrupted at the aqueous/organic interface as the CAS$^-$ readily recombines with H$^+$, resulting in the uncharged, triagonal sugar ester (CAS) and regeneration of Q$^+$Cl$^-$. The triagonal CAS ester is not stable and rapidly releases its sugar to the aqueous phase. By contacting the organic phase with a low pH aqueous medium, the sugars are stripped from the organic phase and the neutral CA is regenerated. The use of HCl as the acid in the stripping solution also allows the regeneration of the lipophilic ion-pair compound, Q$^+$Cl$^-$. Opposite to reactive-extraction, stripping of the sugar causes an increase in the aqueous pH. The moles of H$^+$ removed from the aqueous phase neutralize both CA$^-$ and CAS$^-$ and thus is higher than the moles of sugar released to the strip solution.

Figure 1-7A illustrates the proposed general mechanism of reactive sugar extraction from an aqueous medium. By coupling this scheme to sugar isomerization, the poor extent of the aldose–ketose transformation can be overcome if the sugar extraction is ketose-selective. To achieve this goal, the CA must show a significantly higher binding affinity toward ketose sugars than aldoses under the isomerization conditions. Finally, Figure 1-7B shows the proposed general mechanism for the back-extraction of sugars.
from an organic medium. By varying the acidity of the HCl solution, it is possible to back-extract different concentrations and compositions of sugars into the aqueous phase.

1.2 Objectives and Significance

A mathematical model to predict the results of SIRE-BE will be developed to optimize the process under a variety of operating conditions. Kinetic parameters for the isomerization of aldose to ketose sugars will be determined using enzyme-catalyzed reaction models. Equilibrium constants for the reactive-extraction of sugars into an organic media containing complexing agents (CAs) will be developed based on the theoretical framework presented in Section 1.1.7. Parameters determined from the fits of these two models to experimental data will be used in a comprehensive mathematical model that includes both isomerization and reactive-extraction of aldose and ketose sugars to simulate SIRE. Finally, a mathematical model for the back-extraction of sugars from the organic media will be developed to predict the recovery of sugar.

By conducting SIRE-BE on a xylose solution, a xylulose-rich aqueous sugar media can be produced. This acidic xylulose solution can be easily dehydrated to furfural, a versatile platform chemical. Using existing experimental data for this dehydration reaction, a mathematical model of the reaction kinetics will be developed to predict kinetics of xylulose consumption and furfural production under a range of temperature conditions. This model will also be used to determine which reaction pathways are important in the production of side-products from both furfural and xylulose.
Chapter 2

Enzyme Kinetics

2.1 Introduction

Xylose isomerase (XI, EC 5.3.1.5), (also referred to as glucose isomerase (GXI)), is used to catalyze the isomerization reaction of D-glucose to D-fructose and D-xylose to D-xylulose, respectively. GXI is used commercially in an immobilized form (such as Gensweet® IGI) in the production of high-fructose corn syrup (HFCS). This industrially-used enzyme is produced inside the cells of both the Streptomyces and Bacillus species. Since GXI has a large, established market in the food industry, it can be acquired in bulk and at low cost.

The chemical equilibrium for the sugar isomerization reaction is unfavorable for ketose production, regardless of the catalyst used. This equilibrium limitation is more substantial for xylose isomerization than for glucose; at pH ~7 and 50 °C, the equilibrium ratio of xylulose to xylose is reported as approximately 1:4. Under similar conditions, glucose isomerization to fructose is substantially more favorable with an equilibrium glucose to fructose ratio of approximately 1:1.

The literature suggests that the enzyme-catalyzed isomerization of aldose (substrate) to ketone (product) proceeds via an enzyme intermediate through reversible
reaction pathways. Figure 2-1 shows the mechanism for an enzyme-catalyzed reaction pathway of this type. Previously published mathematical models for sugar isomerization have used Michaelis-Menten kinetics based on this model (Figure 2-1) to predict enzyme behavior under a variety of conditions. However, the Michaelis-Menten model parameters for glucose and xylose isomerization under our experimental conditions of 60 °C and pH 8.5 are unknown.

![Figure 2-1: Enzyme-catalyzed sugar isomerization.](image)

Sugar isomerization has been shown to fit a reversible reaction pathway that proceeds through an enzyme intermediate.

The reversible Michaelis-Menten model derivation is based on the pseudo-steady state formation of the enzyme-substrate/product intermediate. The reaction rate constants shown in Figure 2-1 (k₁, k₂, k⁻¹, k⁻²) can be used to determine the maximum forward and reverse velocities of the sugar isomerization reaction from the enzyme-intermediate. These maximum velocities would occur if all of the enzyme, Eₜ (g/L), is in the intermediate form. Thus, the maximum catalyzed reaction velocities for the forward (Vₘₙ, mol/gₜ*min) and reverse (Vₘᵣ, mol/gₜ*min) reactions can be expressed as shown in Equations 2.1 and 2.2.

\[
Vₘₙ = k₂ [E]ₜ \quad (2.1)
\]
\[
Vₘᵣ = k⁻¹ [E]ₜ \quad (2.2)
\]

The forward and reverse Michaelis constants (kₘₙ and kₘᵣ) represent the relative rates of enzyme-intermediate loss relative to formation via the forward or reverse
pathways. These parameters are shown in Equations 2.3 and 2.4 and both have units of M.

\[
\begin{align*}
    k_{mf} &= \frac{(k_{-1}+k_2)}{k_1} \quad (2.3) \\
    k_{mr} &= \frac{(k_{-1}+k_2)}{k_{-2}} \quad (2.4)
\end{align*}
\]

The four parameters defined in Equations 2.1-2.4 are based solely on the properties of the enzyme-substrate-product system. If the total amount of enzyme and the initial sugar concentration are known, the transient rate of product formation can be predicted.

Since the isomerization reaction does not change the total moles of sugar present, the total concentration of aldose and ketose sugar present at any time must equal the initial concentration of the aldose sugar, \([\text{Aldose}]_0\). At equilibrium, this constraint can be expressed as Equation 2.5.

\[
    [\text{Ketose}]_e = [\text{Aldose}]_0 - [\text{Aldose}]_e \quad (2.5)
\]

At equilibrium, the concentration ratio of ketose to aldose sugar can be represented by \(K_e\) and is shown in Equation 2.6. The concentration of \([\text{Ketose}]_e\) is calculated as the product of the forward velocity \((V_{mf})\) and the reverse Michaelis constant \((k_{mr})\). \([\text{Aldose}]_e\) is found as the product of the reverse velocity \((V_{mr})\) and the forward Michaelis constant \((k_{mf})\). The ratio of these two values yields the overall equilibrium constant, \(K_e\).

\[
    K_e = \frac{[\text{P}]_e}{[S]_e} = \frac{[\text{Ketose}]_e}{[\text{Aldose}]_e} = \frac{k_{mr}V_{mf}}{k_{mf}V_{mr}} \quad (2.6)
\]

\(K_e\) is also predictable based on the values of the four reaction parameters defined in Equations 2.1-2.4. Substituting Equation 2.5 into Equation 2.6 and rearranging for equilibrium aldose sugar concentration results in Equation 2.7.

\[
    [\text{Aldose}]_e = \frac{[\text{Aldose}]_0}{(1+K_e)} \quad (2.7)
\]
The forward and reverse Michaelis-Menten parameters determined in Equations 2.1-2.4 can be used to find the overall maximum reaction velocity, $V_m$, and the overall reaction Michaelis constant, $K_m$, for the reversible sugar transformation. While $V_m$ is actually a constant (Equation 2.8), $K_m$ is not constant. $K_m$ (Equation 2.9) depends on the initial sugar concentration.

$$V_m = \left[1 + \frac{1}{K_e}\right] \frac{k_{mf}V_{mf}}{k_{mr}k_{mf}}$$  \hfill (2.8)

$$K_m = \frac{k_{mf}k_{mr}}{k_{mr}k_{mf}} \left[1 + \frac{1}{k_{mf}} + \frac{K_e}{k_{mr}}\left(\frac{[\text{Aldose}]}{(1+K_e)}\right)\right]$$  \hfill (2.9)

For the reversible, enzyme-catalyzed sugar isomerization reaction, the rate of ketose formation, $r_k$, can be expressed in terms of the overall Michaelis-Menten parameters $V_m$ and $K_m$ for the reaction system as well as the difference between the ketose concentration and its value at equilibrium as shown in Equation 2.10.

$$r_k = -\left[\frac{1}{E_t}\right] \left[\frac{d[\text{Ketose}]}{dt}\right] = \frac{V_m([\text{Ketose}]-[\text{Ketose}]_e)}{K_m+(K_{mf}+[\text{Ketose}]_e)}$$  \hfill (2.10)

To fully characterize the reversible reaction system, four parameters ($V_{mf}$, $V_{mr}$, $k_{mf}$, and $k_{mr}$) must be fit to experimental data. $K_e$, $V_m$ and $K_m$ are calculated from these four parameters and used in Equation 2.10 to predict the rate of product formation during the isomerization reaction.

**2.2 Experimental Methods**

**2.2.1 Experimental materials**

Gensweet® IGI (immobilized xylose isomerase) was a gift from Genencor International, Inc (Palo Alto, CA). This immobilized glucose isomerase is produced from a genetically modified strain of *Streptomyces rubiginosus* and catalyzes the isomerization of glucose to fructose and xylose to xylulose. The Gensweet® pellets were dry, tan-to-
brown, cylinder-shaped granules with a diameter of approximately 0.3-1.2 mm. Gensweet® IGI was stored at 4 °C until use.

Sodium phosphate dibasic, and sodium phosphate monobasic, xylose and glucose were all purchased from Sigma Aldrich (St. Louis, MO).

2.2.2 Sugar isomerization experiments

Transient data was collected in triplicate for the forward (aldose to ketose) sugar isomerization reactions for solutions of either pure glucose (C6) or xylose (C5) with initial concentrations of approximately 82.5mM, 165mM, and 330mM in 50 mM phosphate buffer. Each sugar solution (100 mL) was placed into a capped shake flask and preheated to 60˚C in a shaking water bath. The reaction was initiated (t=0) by adding immobilized enzyme into the flask. The enzyme was allowed to move freely throughout the shake flask for the duration of the experiment. Enzyme loading of 0.45 g/L of sugar solution was held constant for all experiments. Samples (0.5mL) were collected approximately every 10 min for the first hour and every 30 min thereafter for up to 4 hours (a total of 13 samples). Each sample was stored on ice until analysis for both aldose and ketose sugar concentrations.

2.2.3 Analytical methods

Aqueous samples collected from the experiments were diluted as needed and filtered through a 0.22 μm pore-size filter for HPLC. Standards for xylose, xylulose, glucose and fructose were used for HPLC calibrations. All aqueous samples and standards were analyzed using an Agilent 1100 HPLC system equipped with a refractive index detector (RID). Samples were analyzed using two Shodex SH1011 columns (300×8 mm, from Showa Denko K.K, Japan) in series. A mobile phase of 0.05 M H₂SO₄ was run
at 0.6 ml/min; a column temperature of 50 °C and detector temperature of 35 °C were used for optimal peak resolution and detection.

2.2.4 Computational model

Each sugar isomerization experiment was conducted in triplicate with samples collected at the same time points. The concentration of sugar measured at each time point was averaged across the three experiments. The Michaelis-Menten model, Equation 2.10, was fit to the experimental data using a program written in MatLab (see code in Appendix A). The transient isomerization data for each sugar product (ketose) was fit as a complete set (all three concentrations) to determine the best fit for the four model parameters. Based on initial estimates, optimization of \( V_{mf} \), \( V_{mr} \), \( k_{mf} \), and \( k_{mr} \) was achieved using a genetic algorithm. The genetic algorithm, constrained with bounds, obtained a global minimum for the fit of the three sets of experimental data to the integrated ODEs generated with the same initial sugar concentrations. The algorithm randomly selected values for the four parameters within the bounded range and, in a process that mimics biological evolution, adapted each subsequent value to obtain an optimal solution. The sum-squared error (E) between the experimental data and model-predicted values for ketose concentration was calculated as shown in Equation 2.11 for each iteration of the genetic algorithm.

\[
E = \sum_{T=1}^{z} \sum_{t=1}^{x} (\text{[Ketose]}_{\text{model}_{t,T}} - \text{[Ketose]}_{\text{experimental}_{t,T}})^2 \quad (2.11)
\]

In this equation, the inner summation is over \( x \) (usually 13) time points at one initial concentration of sugar and the outer summation is over \( z \) (usually 3) initial concentrations of sugar. A simulation ended when the difference in the sum-squared error
between sequential iterations fell below the established tolerance (10\(^{-6}\)). The error minimization was conducted 10 times for each data set to ensure that the model converged reproducibly.

**2.3 Results**

**2.3.1 Experimental data**

Figure 2-2 shows the experimental results for the isomerization of glucose to fructose at 60°C and pH 8.5 as well as the results of the Michaelis-Menten model fits to the data. The experimental data shows a significant loss of sugar over time that is unaccounted for in the Michaelis-Menten model. The model fits for the fructose product are in very close agreement with the experimental data over all three data sets (Figure 2-2A). The model predictions for glucose concentration over-estimate the amount of glucose actually measured in the reaction media (Figure 2-2B). At equilibrium, the model predictions for glucose concentration differ from the experimental results by approximately 10% for all three initial concentrations. This 10% sugar loss is seen experimentally as the mass balance does not close.

The experimental results for the isomerization of xylose to xylulose at 60°C and pH 8.5 and the model fits to the data are shown in Figure 2-3. Like fructose, the model fits to xylulose concentration data are in close agreement for all three data sets (Figure 2-3A). The actual sugar loss for the C5 isomerization is nearly 15% at equilibrium, as can be seen from the model predictions of xylose in the aqueous media based on a mole balance (Figure 2-3B).
Figure 2-2: Glucose to fructose isomerization experimental time course data and model fit. The average value of the triplicate experimental runs at three initial concentrations ($G_0$) are shown as data points. Experimental error for each data point was ~2%. The computational model is shown as solid lines.
Figure 2-3: Xylose to xylulose isomerization experimental time course data and model fit. The average value of the triplicate experimental runs at three initial concentrations ($X_0$) are shown as data points. Experimental error for each data point was ~2%. The computational model is shown as solid lines.

The values of the four parameters fit to the experimental data, maximum forward and reverse reaction velocities ($V_{mf}$ and $V_{mr}$) and forward and reverse Michaelis-Menten constants ($k_{mf}$ and $k_{mr}$), are shown in Table 2.1 for the isomerization of glucose to fructose and xylose to xylulose. The maximum forward reaction velocity for xylose isomerization was slower than for glucose.
Table 2.1: Forward and reverse Michaelis-Menten constants for sugar isomerization at 60°C and pH 8.5.

<table>
<thead>
<tr>
<th>Initial Sugar</th>
<th>$V_{mf}$ (mol/(g$_{cat}$*min))</th>
<th>$V_{mr}$ (mol/(g$_{cat}$*min))</th>
<th>$k_{mf}$ (M)</th>
<th>$k_{mr}$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>7.10x10^{-4}</td>
<td>5.68x10^{-4}</td>
<td>2.59</td>
<td>2.19</td>
</tr>
<tr>
<td>Xylose</td>
<td>2.31x10^{-4}</td>
<td>5.27x10^{-4}</td>
<td>1.51</td>
<td>1.26</td>
</tr>
</tbody>
</table>

The overall isomerization reaction Michaelis-Menten parameters ($V_m$ and $K_m$) determined from these four parameters are given in Table 2.2. For comparison, values found in the literature measured under other experimental conditions are also included in this table. The overall maximum reaction velocity ($V_m$) for the isomerization of glucose is approximately 3 times faster than that of xylose under our experimental conditions. The dimensionless ratio $K_e$ (calculated from Equation 2.5), which is the ratio of ketose to aldose sugar at equilibrium, is significantly higher for the C6 than the C5 sugars; this indicates that xylose isomerizes to a much lower degree than glucose. These results are

Table 2.2: Overall Michaelis-Menten parameters for aldose to ketose isomerization. The first two rows are those determined from data presented in this chapter. Other parameter values are as reported in the literature.

<table>
<thead>
<tr>
<th>Initial Sugar</th>
<th>Enzyme</th>
<th>pH</th>
<th>Temp (˚C)</th>
<th>$V_m$ (mol/(min*g$_{cat}$))</th>
<th>$K_e$</th>
<th>$K_m$ (M)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Gensweet® IGI</td>
<td>8.5</td>
<td>60</td>
<td>-7.67x10^{-3}</td>
<td>1.06</td>
<td>-12.5[Glucose]$_e$ − 14.39</td>
<td>--</td>
</tr>
<tr>
<td>Xylose</td>
<td>Gensweet® IGI</td>
<td>8.5</td>
<td>60</td>
<td>-4.31x10^{-3}</td>
<td>0.366</td>
<td>-7.2[Xylose]$_e$ − 7.53</td>
<td>--</td>
</tr>
<tr>
<td>Xylose</td>
<td>Sweetzyme, Type Q</td>
<td>6.0</td>
<td>30</td>
<td>-12.5x10^{-4}</td>
<td>0.117</td>
<td>-3.17[Xylose]$_e$ − 1.13</td>
<td>[26]</td>
</tr>
<tr>
<td>Xylose</td>
<td>Sweetzyme, Type Q</td>
<td>7.5</td>
<td>40</td>
<td>-4.14x10^{-4}</td>
<td>0.363</td>
<td>-0.98[Xylose]$_e$ − 0.78</td>
<td>[26]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Sweetzyme IT</td>
<td>7.5</td>
<td>50</td>
<td>-9.26x10^{-4}</td>
<td>0.72</td>
<td>-1.93[Glucose]$_e$</td>
<td>[33]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Sweetzyme IT</td>
<td>7.5</td>
<td>55</td>
<td>-13.8x10^{-4}</td>
<td>0.85</td>
<td>-2.84[Glucose]$_e$</td>
<td>[33]</td>
</tr>
</tbody>
</table>
comparable to those found in the literature (see Table 2.2) under more acidic and lower temperature conditions for other immobilized $GXl$ enzymes\textsuperscript{26,33}. The overall Michaelis constant, which is actually a linear function of equilibrium (or initial) sugar concentration for a reversible reaction, is also given in Table 2.2. To calculate $K_m$ given an initial aldose sugar concentration, the equilibrium concentration of the aldose is first calculated using Equation 2.7.

2.3.2 Empirical model of sugar loss

As shown in Figures 2-2 and 2-3, a small amount of sugar appears to be lost during isomerization. This loss, due either to sugar remaining complexed with $GXl$ or non-specific binding of sugar to the enzyme’s solid support, is not included in the Michaelis-Menten model predictions for aldose concentrations during isomerization. Since accurate predictions for the concentration of both isomers in the aqueous phase are needed to accurately predict reactive-extraction, the transient sugar loss was plotted and fit to an exponential decay. The sugar loss equations, Equations 2.12 and 2.13, were normalized using initial sugar concentration (glucose and xylose, respectively) and total enzyme, and were then used with the transient Michaelis-Menten model predictions of ketose to determine the concentration of aldose sugar remaining in the aqueous reaction media.

$$[G]_{loss} = (-7.0 \times 10^{-4}\ln(time) - 1.2 \times 10^{-3})[G_0]E_t$$ \hspace{1cm} (2.12)

$$[X]_{loss} = (-1.0 \times 10^{-3}\ln(time) - 2.1 \times 10^{-3})[X_0]E_t$$ \hspace{1cm} (2.13)

The new transient Michaelis-Menten model for sugar isomerization which includes empirical sugar loss provides an excellent fit to the transient isomerization data.
The fits of this model for aldose sugar concentrations are shown in Figure 2-4A for glucose and Figure 2-4B for xylose.

**Figure 2-4:** Fit of revised mathematical model to aldose sugar concentration data. The mathematical model of sugar isomerization was revised to include an empirical fit to sugar loss seen experimentally. (A) Glucose and (B) xylose concentrations are shown for the isomerization reactions (same experimental data as Figure 2-2A and 2-3A) isomerizations. The computational model results which includes the empirical function for sugar loss are shown as dashed lines while the original computational model results are shown as a solid line.
2.3.3 Model Validation - Prediction of Isomerization Sugars

The mathematical model developed for prediction of transient sugar concentrations during enzyme-catalyzed isomerization using $GI$ that includes empirical sugar loss was validated by comparing model predictions to additional experimental data. The experimental data, which was collected with an initial sugar concentration of approximately 0.35 M, was collected as described previously. The results of these simulations for glucose and xylose isomerization are shown in Figure 2-5. The model accurately predicts the concentration of all four sugars.

![Figure 2-5: Glucose and xylose isomerization experimental time course data and model predictions](image)

(A) Glucose to fructose. (B) Xylose to xylulose. Model predictions (including the empirical sugar loss term) are shown as solid lines while experimental data is shown as data points.
2.3.4 *GXII* presoak to minimize sugar loss

Additional experiments were conducted for both C6 and C5 sugars to attempt minimize the loss of sugar from either non-specific binding to the enzyme support or sugar that remains complexed to the active site of the *GXII*. Based on information from the enzyme supplier, the sugar may bind non-specifically on the enzyme support. This non-specific binding is expected to occur when dry enzyme is added to sugar solution. To mitigate this loss of sugar, isomerization experiments were also conducted by first presoaking the enzyme in reaction media and then replacing the reaction media with fresh sugar solution.

The immobilized enzyme pellets were soaked for 3 hours at the same experimental conditions as previously described (60°C and pH 8.5) in either a buffered glucose or xylose media. The media was then removed by pipette, leaving behind enough media to keep the *GXII* pellets fully submerged. Fresh sugar media was then added to the submerged pellets. Isomerization time-course data was collected in the manner described in Section 2.2.2. The analytical techniques outlined in Section 2.2.3 were used again to analyze all samples. Finally, kinetic parameters were determined for the Michaelis-Menten model as described in Section 2.2.4.

The results are shown in Figure 2-6 for glucose to fructose isomerization as well as the results of the Michaelis-Menten model fits to the data. The model fits for the fructose product and the glucose substrate are in very close agreement with the experimental data over all three data sets. The experimental data shows a significant improvement in the closure of the mole balance during isomerization. The experimental results for the isomerization of xylose to xylulose at 60°C and pH 8.5 and the Michaelis-
Menten model fits to the data are shown in Figure 2-7. Like the C6 sugar data, the model fits to both the xylose substrate data as well as the xylulose product concentration data are in close agreement for all three data sets. Additionally, the sugar loss for C5 sugars is minimized by the GXI presoak.

Figure 2-6: Glucose to fructose isomerization experimental time course data and model fit for presoaked GXI. The average value of the triplicate experimental runs at three initial concentrations (G₀) are shown as data points. Experimental error for each data point was ~2%. The computational model results are shown as solid lines.
The values of the four parameters fit to the experimental data, maximum forward and reverse reaction velocities and forward and reverse Michaelis-Menten constants, are shown in Table 2.3 for the isomerization of glucose to fructose and xylose to xylulose with GXI presoak. The maximum forward reaction velocity was slower for the isomerization of xylose compared to glucose.

![Graph A](image)

![Graph B](image)

**Figure 2-7: Xylose to xylulose isomerization experimental time course data and model fit for presoaked GXI.** The average value of the triplicate experimental runs at three initial concentrations (X₀) are shown as data points. Experimental error for each data point was ~2%. The computational model results are shown as solid lines.
Table 2.3: Forward and reverse Michaelis-Menten constants for sugar isomerization with GXI presoak at 60°C and pH 8.5.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Initial Sugar</th>
<th>$V_{mf}$ (mol/$(g_{cat}$*min))</th>
<th>$V_{mr}$ (mol/$(g_{cat}$*min))</th>
<th>$k_{mf}$ (M)</th>
<th>$k_{mr}$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh (Dry) Glucose</td>
<td>1.48x10^{-3}</td>
<td>1.13x10^{-3}</td>
<td>4.20</td>
<td>2.82</td>
<td></td>
</tr>
<tr>
<td>Presoaked  Glucose</td>
<td>7.10x10^{-4}</td>
<td>5.68x10^{-4}</td>
<td>2.59</td>
<td>2.19</td>
<td></td>
</tr>
<tr>
<td>Fresh (Dry) Xylose</td>
<td>1.11x10^{-3}</td>
<td>2.33x10^{-3}</td>
<td>2.38</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>Presoaked  Xylose</td>
<td>2.31x10^{-4}</td>
<td>5.27x10^{-4}</td>
<td>1.51</td>
<td>1.26</td>
<td></td>
</tr>
</tbody>
</table>

2.4 Discussion

In this chapter, the Michaelis-Menten model for the reversible enzyme-catalyzed sugar isomerization was presented and fit to experimental data collected from aldose sugar isomerization. The mathematical model, which used four parameters, fit the experimental data for transient ketose concentration very well for all three initial sugar concentrations. Reduced reaction velocities and slower overall kinetic profile were observed for isomerization of xylose compared to glucose.

The equilibrium ratio of xylulose to xylose was determined for higher temperatures (60°C) and more basic reaction media (pH 8.5) than previously reported. Aldose sugar concentrations calculated from the model predictions of ketose sugar concentrations using a mole balance over-estimated the measured concentration of aldose sugar in the aqueous solution. The experimentally-observed sugar loss, which followed an exponential decay, can possibly be attributed to sugar that remains in the sugar-enzyme intermediate form (AE or KE in Figure 2-1) or non-specific binding of sugar to the enzyme pellets. This observed sugar loss can be mitigated by pre-soaking the GXI in aqueous sugar media. This presoak reduces the experimentally observed sugar loss to
~3%. The kinetic parameters shown in Table 2.3 can be used in subsequent models where the enzyme is either fresh (dry) or presoaked and/or used for multiple batches.

The kinetic Michaelis-Menten models can be used to approximate the transient sugar isomer profiles for a variety of initial sugar concentrations and enzyme loadings. The empirical sugar loss equations were derived assuming a direct dependency on enzyme loading, but no experimental data was collected to verify this relationship. Additionally, the transient Michaelis-Menten model can be used to predict the length of time needed for the isomerization reaction to reach equilibrium. The mathematical model could also be expanded to include temperature and pH-dependencies of the Michaelis-Menten model parameters to predict isomerization outcomes under a variety of temperatures and pH conditions to create a more robust understanding of the enzyme. Arrhenius-like dependencies for varied temperatures have been reported previously in the literature for immobilized xylose isomerase.

The next step in developing a mathematical model that predicts the outcome of simultaneous sugar isomerization and reactive-extraction is the development of a reactive-extraction model that predicts aldose and ketose partitioning between organic and aqueous phases. The transient, reversible enzyme-catalyzed isomerization model can be used in conjunction with a reactive-extraction model (or another mathematical model of product removal) to predict the efficacy of these strategies to shift the extent of the isomerization reaction and further enhance the production of ketose sugars.
Chapter 3

Reactive Extraction

3.1 Introduction

Borate and boronic acids have been reported to preferentially binding to ketose sugars\textsuperscript{11,15,16}. Figure 3-1 shows the mechanism for the binding of borate to sugar molecules. Triagonalboric acid, the native form of borate, is a weak-acid (pK\textsubscript{a} > 9)\textsuperscript{16}. Boronic acid binds with sugar through a dehydration reaction to form a triangular ester. However, this type of ester complex is not stable. At neutral to alkaline pH, borate, in the form of tetrahydroxyborate, is able to form a stable ester with sugar.

\begin{align*}
\text{Triagonalboric acid} + \text{Sugar} & \rightleftharpoons \text{Triangular Ester} \quad \text{(Unstable)} \\
\text{Tetrahydroxyborate ion} + \text{Sugar} & \rightleftharpoons \text{Tetragonal Ester} + \text{H}_{2}\text{O} 
\end{align*}

Figure 3-1: The mechanism for borate binding to the sugar.
Ketose sugars, such as xylulose, are typically more readily converted to useful products and have a higher market value compared to aldose sugars (based on Sigma Aldrich catalog pricing). Simultaneous-isomerization-and-reactive-extraction (SIRE) followed by back-extraction (BE) can be used to produce high-purity ketose solutions. During SIRE, ketose sugars are selectively removed from the aqueous reaction mixture by binding to a lipophilic boronic acid, or complexing agent (CA), confined to an immiscible organic phase. Selective removal of the ketose sugar to the organic phase shifts the unfavorable isomerization equilibrium to allow more xylulose production. The addition of a lipophilic amine salt, $Q^+Cl^-$, to the organic phase leads to ion-pairing between the salt cation and the negatively charged CA and increases sugar transfer. The proposed reactions for SIRE are illustrated in Figure 3-2. Under slightly alkaline conditions, the complexing agent (CA$_{org}$) reacts with water and is converted to a tetragonal charged form (CA$^-_{i}$) releasing H$^+$ into the aqueous phase. Both forms of CA, CA$_{org}$ and CA$^-_{i}$, can bind with the sugar to form CAS$_{org}$ and CAS$_{i}$, respectively. Either of the charged boronic acid species, CA$^-_{i}$ or CAS$^-_{i}$, can be stabilized by ion-pair formation with $Q^+Cl^-$. When ion pairing occurs, the CA species become charge-neutral as CAS$^+_{org}Q^-_{org}$ or CA$^-_{org}Q^+_{org}$. Chloride is assumed to be transferred into the aqueous phase to maintain charge neutrality.

In this chapter, a mathematical model of reactive-extraction is presented. Experiments were conducted to calculate the model parameters used in the model. Changes in pH during reactive-extraction can be measured to determine the amount of H$^+$ released, indicating CA that has been converted to a negatively-charged form. In the experiments described here the pH is buffered, so the pH is repeatedly titrated back to the
initial pH of 8.5 to determine the amount of H\(^+\) released and to ensure that the aqueous phase buffering capacity is unchanged throughout the experiment. Changes in sugar concentration in the aqueous phase were measured by HPLC to determine the amount of sugar extracted into the organic phase. Finally, the change in Cl\(^-\) concentration in the aqueous phase associated with stabilization of the charged CA species with by ion-pair formation with Q\(^+\) was measured by using a chloride-selective ion probe. The model parameters, once determined, were used in the equilibrium reactive-extraction model to predict reactive-extraction outcomes of sugar isomers.

### 3.2 Experimental methods

By contacting aqueous and organic phases and measuring species concentrations at equilibrium, equilibrium constants for the pathways shown in Figure 3-2 were determined.

**Figure 3-2: Proposed pathway of SIRE.** CA species found in the organic phase are shown in red while green species of CA are confined to the aqueous-organic interface. All aqueous species are shown in blue. The equilibrium constants are shown in black. When an organic phase containing CA is placed into contact with an alkaline aqueous phase, the CA becomes charged and is confined to the phase interface (CA\(^-\)\(_i\)). H\(^+\) is transferred to the aqueous phase. When sugar is present in the aqueous phase (S\(_{aq}\)), the sugar can form a triagonal ester (CAS\(_{org}\)) or a tetragonal ester confined to the aqueous-organic interface (CAS\(_i\)). When a lipophilic amine salt (Q\(^+\)Cl\(^-\)) is added to the organic phase, the cation (Q\(^+\)) can ion-pair with the negatively charged CA species, (CA\(^-\)\(_i\) or CAS\(_i\)), transferring the salt anion (Cl\(^-\)) into the aqueous phase.
The pH was measured and titrated back to the initial pH of 8.5 every 15 min. Equilibrium was assumed reached when the pH stabilized for two consecutive measurements (~30 min). All experiments were conducted in triplicate. By isolating each component of the system, the equilibrium dissociation and binding constants were determined independently. The experiments designed to determine the equilibrium constants for reactive-extraction are summarized in Table 3.1. The aqueous phase media was a sodium phosphate buffer (50mM) and the organic phase was octanol. Naphthalene-2-boronic acid (N2B) was the CA used for all experiments. Aliquat® 336 was the ion pairing salt (Q⁺Cl⁻) used in the organic phase.

Table 3.1: Summary of experiments to determine equilibrium parameters for reactive-extraction (RE) at pH 8.5 and 60°C. The aqueous phase was 50 mM sodium phosphate buffer with sugar added for select experiments. The organic phase (octanol) contained 0.165 M N2B and/or 0.413 M Aliquat® 336 for reactive-extraction experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Aqueous Phase Additives</th>
<th>Organic Phase Additives</th>
<th>Measure</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N/A</td>
<td>N2B</td>
<td>OH⁻ added</td>
<td>K₁</td>
</tr>
<tr>
<td>2</td>
<td>N/A</td>
<td>N2B, Aliquat® 336</td>
<td>OH⁻ added [Cl⁻]aq</td>
<td>K₅</td>
</tr>
</tbody>
</table>

In the following subsections, experimental methods for data collection are described for each of the experiments listed in Table 3.1. Analytical methods are also described for measurements of aqueous phase sugar and chloride ion concentrations. The
last section describes the mathematical analysis of the data to calculate the equilibrium parameters (K’s).

3.2.1 Experimental methods for Experiment 1

To determine the equilibrium acid dissociation constant for the N2B (K₁), equal volumes (50mL) of an aqueous phase containing 50 mM phosphate buffer at pH 8.5 and an organic phase containing 0.165 M N2B were contacted in a covered 100 mL Erlenmeyer flask and maintained at 60˚C in a shaker water bath. The pH of the aqueous phase was measured every 15 min, and the pH was recorded. Sodium hydroxide (1-10 M NaOH, depending on pH change) was used to titrate the pH back to 8.5, and the concentration and volume of NaOH added to the aqueous phase was recorded. This process was repeated until the pH remained at approximately 8.5 for two consecutive measurements (~30min) at which point the system was assumed to be at equilibrium.

3.2.2 Experimental methods for Experiment 2

To determine the equilibrium constant (K₅) for the ion pairing of the conjugate base N2B anion with the lipophilic salt cation, equal volumes (50 mL) of an aqueous phase containing 50 mM phosphate buffer at pH 8.5 and an organic phase containing 0.165 M N2B and 0.413 M Aliquat® 336 (Q⁺Cl⁻) were contacted in a covered 100 mL Erlenmeyer flask and maintained at 60˚C in a stirred water bath. The pH of the aqueous phase was measured every 15 min, and the change in pH recorded. Sodium hydroxide (NaOH) was used to titrate the pH to 8.5, and the amount of NaOH added to the aqueous phase was recorded. The reaction mixture was considered at equilibrium when the pH remained at approximately 8.5 for two consecutive measurements (~30 min). The aqueous and organic phases were separated and the aqueous layer was reserved for
chloride measurement. Total moles of OH\textsuperscript{−} added was calculated and [Cl\textsuperscript{−}]\textsubscript{aq} at equilibrium was measured.

3.2.3 Experimental methods for Experiment 3

The equilibrium constants for CA binding with sugar (K\textsubscript{2}, K\textsubscript{3}, K\textsubscript{4}) and the ion pairing of the tetragonal bound to sugar (CAS\textsuperscript{−}) with Q\textsuperscript{+}Cl\textsuperscript{−} (K\textsubscript{6}) were found by contacting equal volumes (50 mL) of aqueous phase containing 50 mM phosphate buffer at pH 8.5 containing 0.165 M of a single sugar (glucose, fructose, xylose or xylulose) with an organic phase containing 0.165 M N2B and 0.413 M Aliquat\textsuperscript{®} 336 (Q\textsuperscript{+}Cl\textsuperscript{−}). The experiments were conducted in the same way as Experiment 1. The experiments continued until the pH remained at approximately 8.5 for two consecutive measurements (~30 min). The aqueous and organic phases were centrifuged at 5,000 rpm for 5 min at room temperature, separated, and the aqueous layer was reserved for chloride measurements. Total moles OH\textsuperscript{−} added was calculated, and the final [Cl\textsuperscript{−}]\textsubscript{aq} was measured. A sample of the aqueous phase was also analyzed for sugar concentration by HPLC.

3.2.4 Analytical methods for aqueous sugar

Aqueous samples collected from the experiments were diluted as needed and filtered through a 0.22 μm pore-size filter for HPLC. Standards for xylose, xylulose, glucose and fructose were used for HPLC calibrations. All aqueous samples and standards were analyzed using an Agilent 1100 HPLC system equipped with a refractive index detector (RID). Samples were analyzed using two Shodex SH1011 columns (300×8 mm, from Showa Denko K.K, Japan) in series. A mobile phase of 0.05 M H\textsubscript{2}SO\textsubscript{4} was run.
at 0.6 ml/min; a column temperature of 50 °C and detector temperature of 35 °C were used for optimal peak resolution and detection.

### 3.2.5 Analytical methods for aqueous chloride

Chloride measurements were determined at room temperature using a Cole Parmer Combination Ion Selective Chloride Electrode (Vernon Hills, Illinois) attached to a pH meter set to mV mode. An ion strength adjuster (ISA) containing 5M NaNO₃ was added to each sample at a rate to achieve an ionic strength of approximately 0.1M. A chloride standard (NaCl) was used to calibrate the internal standard of an Orion™ Star A211 benchtop meter (Thermo Scientific, Waltham, WA) to correlate millivolts (mV) to chloride concentration.

### 3.2.6 Equilibrium parameter determination and mathematical model

Each of the experiments shown in Table 3.1 and described in the previous subsections was conducted in triplicate. To determine the best fit of the model equations to the experimental data, the average values for concentrations of sugars in the aqueous and the organic layers, chloride in the aqueous layer, and moles of NaOH added to the system to maintain the pH at 8.5 were used. The equations used to determine the equilibrium constants, shown in Equations 3.1-3.7, are based on the proposed reaction pathways in Figure 3-2. Equations 3.4 and 3.7 reflect that the equilibrium constants are part of two reversible cycles and are, therefore, not all independent.

\[
K_1 = \frac{[C\text{A}^-][H^+]_{\text{aq}}}{[C\text{A}]_{\text{org}}} \quad (3.1)
\]

\[
K_2 = \frac{[C\text{A}S^-][H^+]_{\text{aq}}}{[C\text{A}S]_{\text{org}}} \quad (3.2)
\]

\[
K_3 = \frac{[C\text{A}]_{\text{org}}}{[C\text{A}]_{\text{org}}[S]_{\text{aq}}} \quad (3.3)
\]
In addition to the equilibrium relations, mole balances on the different species were used as constraining equations. Equations 3.8, 3.9 and 3.10 are based on conservation of mass (moles) for complexing agent, sugar, and chloride, all of which can be located in either phase.

\[
\begin{align*}
CA_{total} &= CA_{org} + CA_i^- + CAS_{org} + CAS_i^- + CA^- Q_{org}^+ + CAS^- Q_{org}^+ \\
Sugar_{total} &= CAS_{org} + CAS_i^- + CAS^- Q_{org}^+ + S_{aq} \\
Cl^-_{total} &= Q^+ Cl^-_{org} + Cl^-_{aq}
\end{align*}
\]

Equation 3.11 is a constraint based on the assumption that H\(^+\) ion is only transferred to the aqueous phase when the weak acid CA is converted to its negatively charged conjugate base form. Although the aqueous phase is buffered, NaOH is added to titrate the pH back to the initial pH of 8.5, so the buffering capacity of the aqueous phase remains unchanged. Thus, the amount of H\(^+\) released to the aqueous phase should be exactly balanced by OH\(^-\) added during the titrations.

\[
OH^-_{added} = CA_i^- + CAS_i^- + CA^- Q_{org}^+ + CAS^- Q_{org}^+
\]
The last constraining equation used, Equation 3.12, is based on the assumption that when the lipophilic $Q^+\text{Cl}^-$ dissociates for ion pairing, the chloride ion is not soluble in the organic phase and transfers to the aqueous phase.

$$\text{Cl}^-_{\text{aq}} = \text{CA}^-\text{Q}_{\text{org}} + \text{CAS}^-\text{Q}_{\text{org}}$$  \hspace{1cm} (3.12)

The ionization of CA was determined using the data from Experiment 1 (N2B, no Aliquat\textsuperscript{®}336 or sugar) and Equation 3.1. With no sugar present, the moles of $H^+$ released by the CA and the moles of $\text{CA}_i^-$ are both assumed equal to the moles of $\text{OH}^-$ added to the aqueous phase. The amount of uncharged CA remaining, $\text{CA}_{\text{org}}$, was determined based on a mole balance (Equation 3.8 with no sugar or ion-paired species).

$K_5$ was determined using $K_1$ calculated from Experiment 1 and the data collected in Experiment 2 (N2B and Aliquat\textsuperscript{®}336, no sugar) from Equations 3.1 and 3.5. The chloride remaining in the organic phase ($Q^+\text{Cl}^-_{\text{org}}$) was calculated using Equation 3.10 with the measured amount of chloride in the aqueous phase ($\text{Cl}^-_{\text{aq}}$).

The data collected in Experiment 3A-D were used to determine the equilibrium constants $K_2$, $K_3$, $K_6$ for each sugar by solving a matrix of 10 equations (Equations 3.1-3.3, 3.5-3.6, and 3.8-3.12) and 10 unknowns (see Appendix B). The concentration of sugar in the organic phase was calculated assuming closure of the mass balance (Equation 3.9). Equilibrium parameters for $K_4$ and $K_7$ were calculated after the matrix was solved.

A computer program was written in MatLab using the equilibrium parameters to simulate reactive-extraction (see Appendix B). These simulations were used to determine the concentrations of all of the participating species as well as the partitioning of sugar.
and chloride between the organic and aqueous phases at equilibrium. Results of the model simulations were compared to additional experimental data for model validation.

3.3 Results

3.3.1 Experimental data

Table 3.2 summarizes the data collected for each of the experiments (Table 3.1) and the average values used to fit the parameter values in the equilibrium reactive-extraction model described in the previous section. The standard deviation for all values was less than 2%. Between Experiment 1 and Experiment 2, a nearly 150 fold increase in \( \text{OH}^- \) added to the aqueous phase to titrate the pH change was required indicating that the majority of the charged CA resides in an ion-pair with \( Q^+ \). Ketose sugars were extracted more readily than their aldose counterparts as significantly less sugar remained in the aqueous phase for the xylulose and fructose experiments. Additionally, higher concentrations of chloride were observed in the aqueous layer for the ketose sugar experiments, indicating that more of the N2B is ion-paired with \( Q^+ \) and complexed with sugar in the organic phase.

The equilibrium constants at 60°C for N2B, Aliquat®336, and the four biomass sugars are summarized in Table 3.3. Based on the experimental data and Equation 3.1, the pK\text{a1} for the ionization of N2B is 10.8. This is in agreement with the values assumed in the literature for boronic acids like N2B (pK\text{a} > 9)\textsuperscript{11,12,14}. The pK\text{a2}'s for the N2B-sugar esters (K\textsubscript{2}) are lower than that of N2B alone, another trend observed in the literature for boronic acids\textsuperscript{35}. The pK\text{a2} for the aldose sugars are approximately 9 while the pK\text{a2} of the ketose sugars are significantly lower at 1-2 pH units below that of aldose sugars. The significance of this is that at a pH equal to the pK\text{a2}, each of the sugars will be extracted
Table 3.2: Experimental results for reactive-extraction experiments averaged over triplicate experiments. The concentration of sugar in the organic phase was determined by closure of the mass balance. The standard deviation for the triplicate data for all experiments was less than 2%.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>pH</th>
<th>[Sugar], M</th>
<th>OH⁻ added (mol)</th>
<th>Cl⁻ Final (mol)</th>
<th>Average [Sugar], M</th>
<th>Average OH⁻ added (mol)</th>
<th>Average Cl⁻ Final (mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Aq Initial</td>
<td>Aq Final</td>
<td>Org Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>1</td>
<td>8.51</td>
<td>8.64</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4.80x10⁻³</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>8.51</td>
<td>8.63</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4.75x10⁻³</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>8.51</td>
<td>8.63</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4.75x10⁻³</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>8.52</td>
<td>8.53</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.41x10⁻³</td>
<td>7.06x10⁻³</td>
</tr>
<tr>
<td></td>
<td>8.52</td>
<td>8.54</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.45x10⁻³</td>
<td>7.04x10⁻³</td>
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<td></td>
<td>8.52</td>
<td>8.55</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.50x10⁻³</td>
<td>7.05x10⁻³</td>
</tr>
<tr>
<td>3A</td>
<td>8.52</td>
<td>8.51</td>
<td>0.163</td>
<td>0.108</td>
<td>0.055</td>
<td>6.16x10⁻³</td>
<td>5.95x10⁻³</td>
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<td>Glucose</td>
<td>8.52</td>
<td>8.52</td>
<td>0.163</td>
<td>0.110</td>
<td>0.053</td>
<td>6.24x10⁻³</td>
<td>6.06x10⁻³</td>
</tr>
<tr>
<td></td>
<td>8.52</td>
<td>8.52</td>
<td>0.163</td>
<td>0.115</td>
<td>0.048</td>
<td>6.20x10⁻³</td>
<td>6.01x10⁻³</td>
</tr>
<tr>
<td>3B</td>
<td>8.51</td>
<td>8.49</td>
<td>0.156</td>
<td>0.049</td>
<td>0.107</td>
<td>7.20x10⁻³</td>
<td>6.55x10⁻³</td>
</tr>
<tr>
<td>Fructose</td>
<td>8.51</td>
<td>8.50</td>
<td>0.156</td>
<td>0.054</td>
<td>0.102</td>
<td>7.30x10⁻³</td>
<td>6.70x10⁻³</td>
</tr>
<tr>
<td></td>
<td>8.51</td>
<td>8.50</td>
<td>0.156</td>
<td>0.051</td>
<td>0.105</td>
<td>7.26x10⁻³</td>
<td>6.80x10⁻³</td>
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<tr>
<td>3C</td>
<td>8.53</td>
<td>8.51</td>
<td>0.160</td>
<td>0.102</td>
<td>0.058</td>
<td>6.44x10⁻³</td>
<td>6.11x10⁻³</td>
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<tr>
<td>Xylose</td>
<td>8.53</td>
<td>8.50</td>
<td>0.160</td>
<td>0.111</td>
<td>0.049</td>
<td>6.41x10⁻³</td>
<td>6.15x10⁻³</td>
</tr>
<tr>
<td></td>
<td>8.53</td>
<td>8.52</td>
<td>0.160</td>
<td>0.104</td>
<td>0.056</td>
<td>6.52x10⁻³</td>
<td>6.10x10⁻³</td>
</tr>
<tr>
<td>3D</td>
<td>8.49</td>
<td>8.48</td>
<td>0.161</td>
<td>0.021</td>
<td>0.140</td>
<td>7.85x10⁻³</td>
<td>6.99x10⁻³</td>
</tr>
<tr>
<td>Xylulose</td>
<td>8.49</td>
<td>8.50</td>
<td>0.161</td>
<td>0.030</td>
<td>0.131</td>
<td>7.92x10⁻³</td>
<td>7.07x10⁻³</td>
</tr>
<tr>
<td></td>
<td>8.49</td>
<td>8.47</td>
<td>0.161</td>
<td>0.025</td>
<td>0.136</td>
<td>7.86x10⁻³</td>
<td>6.95x10⁻³</td>
</tr>
</tbody>
</table>
to the same extent by the N2B. Thus, ketose sugar extractions can be successfully accomplished at much lower pH values (i.e. pH 8.5) unlike the pH of 11 used in the literature for aldose sugar extractions\textsuperscript{11,14}.

**Table 3.3: Equilibrium parameters for reactive-extraction.** The equilibrium parameters were determined from the averaged data for each experiment given in Table 3.2 and Equations 3.1-3.12.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$K_1$ (M)</th>
<th>$K_2$ (M)</th>
<th>$K_3$ (M$^{-1}$)</th>
<th>$K_4$ (M$^{1}$)</th>
<th>$K_5$ (unitless)</th>
<th>$K_6$ (unitless)</th>
<th>$K_7$ (M$^{1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.34x10$^{-11}$</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.01x10$^3$</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3A Glucose</td>
<td>--</td>
<td>4.47x10$^{-10}$</td>
<td>2.28x10$^1$</td>
<td>7.61x10$^2$</td>
<td>--</td>
<td>2.13x10$^0$</td>
<td>1.62x10$^0$</td>
</tr>
<tr>
<td>3B Fructose</td>
<td>--</td>
<td>27.3x10$^{-10}$</td>
<td>3.64x10$^1$</td>
<td>74.1x10$^2$</td>
<td>--</td>
<td>4.22x10$^0$</td>
<td>31.1x10$^0$</td>
</tr>
<tr>
<td>3C Xylose</td>
<td>--</td>
<td>9.03x10$^{-10}$</td>
<td>1.97x10$^1$</td>
<td>13.3x10$^2$</td>
<td>--</td>
<td>1.63x10$^0$</td>
<td>2.16x10$^0$</td>
</tr>
<tr>
<td>3D Xylulose</td>
<td>--</td>
<td>124x10$^{-10}$</td>
<td>4.69x10$^1$</td>
<td>435x10$^2$</td>
<td>--</td>
<td>4.18x10$^0$</td>
<td>181x10$^0$</td>
</tr>
</tbody>
</table>

Interestingly, fructose has a 6-fold higher $K_2$ than glucose, and xylulose has a 13.7-fold higher $K_2$ than xylose. This indicates that in single sugar isomerization and reactive-extraction, xylulose is more easily separated from its isomer than fructose. In comparison, the $K_2$ for xylulose is more than 4.5 times higher than that of fructose, indicating that it is the most readily-extractable of the four sugars.

Additionally, $K_4$ and $K_5$ are extremely large relative to the other values. This is consistent with the assumption that $CA_i^-$ is very unstable at the interface and comprises an insubstantial amount of the total complexing agent total (<0.1%).

Finally, a sensitivity analysis was performed in order to determine the level of uncertainty in the equilibrium constants. This analysis revealed that the parameters shown in Table 3.3 are extremely sensitive to a ±25% change in each of the individual parameters with the exception of the equilibrium constant for the disassociation of the
Due to the sensitivity of the calculated parameters, it is essential to validate the model with additional experimental data.

3.3.2 Model Validation – Prediction of RE with mixed sugars

To validate the equilibrium model of reactive extraction and the parameter values calculated from the experimental data, MatLab simulations were used to predict sugar extraction from mixtures of sugar isomers. The sugar extraction experiments were conducted using the same organic phase composition (0.165 M N2B and 0.413 M Aliquat® 336 in octanol), pH (8.5) and temperature (60°C) as prior experiments; concentrations of the sugar isomer mixtures used for model validation are shown in Table 3.4. The sugar extractions predicted by the model simulations were compared to the experimental data collected.

Table 3.4: Mixed sugar reactive-extraction validation experimental conditions. The organic phase was octanol containing 0.165 M N2B and 0.413 M Aliquat® 336. The aqueous phases all contained 50 mM sodium phosphate buffer at pH 8.5. The phases were maintained at 60°C.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Initial Sugar (Aqueous)</th>
<th>Aldose (M)</th>
<th>Ketose (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Glucose/Fructose</td>
<td>0.083</td>
<td>0.083</td>
</tr>
<tr>
<td>B</td>
<td>Xylose/Xylulose</td>
<td>0.083</td>
<td>0.083</td>
</tr>
<tr>
<td>C</td>
<td>Glucose/Fructose</td>
<td>0.11</td>
<td>0.055</td>
</tr>
<tr>
<td>D</td>
<td>Xylose/Xylulose</td>
<td>0.11</td>
<td>0.055</td>
</tr>
</tbody>
</table>

The mixed sugar extraction experiments were conducted as described in the Experimental Methods for Experiment 3 (Section 3.2.3), with data collected for the moles of OH− added, aqueous chloride and sugar concentrations, and final pH.

Figure 3-3 shows the results of the mixed sugar reactive-extraction experiments and model simulations. The simulations closely predict the experimental results for
partitioning for both C5 and C6 sugars with different initial ratios of aldose and ketose sugars. In addition, the chloride transfer and OH$^-$ addition predicted by the simulations are in very close agreement to those measured experimentally. These reactive-extraction experiments validate the parameters calculated and the model developed for reaction-extraction of C5 and C6 sugar isomers.

**Figure 3-3: Model predictions and experimental results for mixed sugar reactive-extraction.** Initial sugar concentrations are shown for each mixture of sugar isomers. Panels A and B show the results of reactive-extraction for an initial concentration ratio of 1:1 aldose:ketose. Panels C and D show the results for an initial ratio of 2:1 aldose:ketose. Bars represent experimental values; squares are the corresponding model simulation results. The sugar:N2B ratio was 1:1 and the N2B:Aliquat®336 ratio was 1:2.5 in all experiments.
Figure 3-4 shows model predictions of the distribution of CA among the nine different forms possible with two sugar reactive-extraction. A large percentage of the CA (N2B) is predicted to be ion paired to Q\(^+\) but not attached to sugar.

Figure 3-4: Model Predictions of [CA\(_{\text{species}}\)] from simulations (Figure 3-3). Panel A shows the model predictions of the concentrations of CA species for the two initial ratios of glucose:fructose shown in Figure 3-3 A, C. Panel B shows the same predictions for the experiments shown in Figure 3-3 B, D.
3.4 Discussion

The mathematical model developed for the reactive-extraction of sugars from aqueous media into organic media accurately predicts equilibrium sugar extraction under a variety of experimental conditions. The model, which uses equilibrium dissociation and binding constants measured experimentally for an octanol organic phase containing the boronic acid N2B and the ion-pairing salt Aliquat®336, can simulate reactive-extraction of single sugars and sugar mixtures. The model is also able to accurately predict the moles of OH\(^-\) that must be added to maintain a constant pH during reactive-extraction and the moles of Cl\(^-\) transferred to the aqueous media that results from ion-pairing in the organic phase.

Experimentally, the ketose forms of the C6 and C5 sugars were preferentially extracted into the organic phase as shown in Figure 3-3. The values calculated for the acid dissociation constants reflect significantly lower pK\(_{a2}\) values for the ketose sugars. The lower pK\(_{a2}\) values allow for significant quantities of ketose sugar to complex with the N2B at pH 8.5, the highest pH that can be used if combining RE with isomerization of sugars with the GXI enzyme. The differential partitioning of aldose and ketose sugars is necessary to allow for enhanced isomerization of aldose to ketose and separation of sugars from hydrolysates in their ketose forms.

The distribution of the various species of the CA predicted by the RE model at equilibrium (Figure 3-4, same conditions as Figure 3-3) indicate that at 60°C and pH 8.5, much of the N2B present in the organic phase is ion-paired to Q\(^+\) but unattached to sugar (light orange portion of the bar). This is reflected in the large equilibrium constant K\(_S\) determined in the parameter fitting. From a practical standpoint, this indicates that much
of the base added to maintain pH 8.5 is consumed without extraction of sugar and represents an added cost to the process.

It is unknown how sensitive the reactive-extraction parameters determined here are to changes in pH, temperature, and organic phase composition. The experiments outlined in Table 3.1 can be used as a framework to quickly screen new complexing agents, lipophilic salts, and organic media compositions for use in a reactive-extraction system. By collecting data at extremes of the conditions likely to be encountered in a reactive-extraction system and calculating model parameters for these cases, a sensitivity analysis could be performed to determine which of the parameters is most-sensitive to changes in the reactive-extraction system and the resulting impact on sugar extraction performance.

The next step in the modeling is to combine reactive-extraction with sugar isomerization. Experimentally, reactive-extraction occurs quickly relative to isomerization, therefore it may be considered at pseudo-steady state. Combining the enzyme kinetic models (Chapter 2) with the equilibrium reactive-extraction model demonstrated in this chapter will allow the development of a kinetic model to simulate simultaneous isomerization of aldose to ketose sugars and reactive-extraction. Chapter 4 describes the development of this model.
Chapter 4

Simultaneous Isomerization and Reactive-Extraction followed by Back-Extraction

4.1 Introduction

Reactive-extraction strategies are reported in the literature to separate aldose sugars from aqueous solutions: xylose from a hemicellulose hydrolysate\textsuperscript{36} and glucose and xylose from ionic liquid/water mixtures\textsuperscript{14}. These two studies successfully validated that sugars can be removed by a reactive-extraction approach, achieving adequate extraction efficiencies under highly alkaline conditions (pH of 11). However, these studies required high concentrations of complexing agent (boronic acid, N2B) in the organic phase (7:1 ratio of complexing agent to sugar). The principal reason for the extreme alkalinity requirement is the low affinity of the aldose sugars to the complexing agents. Under these pH conditions, sugar degradation becomes a serious concern.

Ketose sugars are extracted with a much higher affinity than aldose sugars. The aldose sugars found in biomass hydrolysate can be isomerized to their ketose counterparts, glucose to fructose and xylose to xylulose. This isomerization is catalyzed by the enzyme xylose isomerase ($GXI$). Industrially, the high fructose corn syrup industry
has been using commercially available immobilized \textit{GXI} such as Sweetzyme\textsuperscript{®} and Gensweet\textsuperscript{®} for glucose to fructose transformation. With an optimal pH of activity between pH 7-8.5, immobilized \textit{GXI} can effectively be employed to transform biomass hydrolysate at the operating conditions of reactive-extraction of ketose sugars\textsuperscript{37}. The reactive-extraction serves a product removal role to effectively force the isomerization toward the ketose product, thereby overcoming the inherently poor equilibrium of the isomerization reaction. Developing a predictive mathematical model coupling isomerization and the reactive-extraction will be useful for quickly determining the effects of changing ratios of components on the overall isomerization and RE efficiencies.

In this chapter, a comprehensive model of SIRE is used to predict outcomes from RE of aldose sugars. By combining the enzyme kinetic parameters determined in Chapter 2 for the Michaelis-Menten isomerization model and the RE equilibrium constants in Chapter 3 for the equilibrium RE model, the overall predictive model was developed to simulate SIRE. This model was then validated against experimental SIRE data. Finally, model predictions were generated for a variety of SIRE scenarios.

4.2 Experimental methods and materials

The procedure for SIRE-BE was modified from Li, et al.\textsuperscript{37}. The SIRE experiments were conducted in a continuous reactor-type system. Figure 4-1 shows the experimental equipment. A 1 L hollow fiber membrane liquid/liquid contacting system was used. Pumps (pump heads: FF-00198-PQ, FF-00198-PS, Cole Parmer, Vernon Hills, Illinois) are used to move the aqueous and organic phases cocurrently through a hollow fiber membrane contactor (HFMC) (Liqui-Cel 2.5x8 membrane contactor, Quantum
Flow Technologies, Fort Mill, SC). The pH of the aqueous phase was maintained using the Easy pH Titrator, (Mettler Toledo, Columbus, OH). The temperature of both the aqueous and organic phases was controlled by hot plates which heated the fluid reservoirs.

![Image of SIRE system experimental set-up](image)

**Figure 4-1: SIRE system experimental set-up.** The aqueous and organic phases are contacted across a hollow fiber membrane. The two immiscible phases are pumped in a cocurrent fashion.

Gensweet® IGI (immobilized xylose isomerase) was a gift from Genencor International, Inc (Palo Alto, CA). This immobilized xylose isomerase is produced from a genetically modified strain of *Streptomyces rubiginosus* and catalyzes the isomerization of glucose to fructose and xylose to xylulose. The Gensweet® pellets were dry, tan-to-brown, cylinder-shaped granules with a diameter of approximately 0.3-1.2 mm. Gensweet® IGI was stored at 4 °C until use. Xylulose was purchased from zuChem Inc. (Chicago, IL). Napthelene-2-boronic acid (N2B), and 1-octanol were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA). Additional chemicals, including xylose,
Aliquat® 336, sodium phosphate dibasic, and sodium phosphate monobasic were all purchased from Sigma Aldrich (St. Louis, MO).

The experiments conducted are summarized in Table 4.1. The aqueous phase (50 mM sodium phosphate buffer, pH 8.5) contained either glucose or xylose. The organic phase (octanol) consisted of different ratios of Aliquat® 336 to N2B (1.5:1, 2:1 and 2.5:1). The aqueous and organic phase volumes were held constant for all experiments (750 mL).

**Table 4.1: Summary of aqueous and organic phase initial compositions used for SIRE-BE simulations and experiments.** The combinations of initial conditions shown below were used for both C6 (glucose) and C5 (xylose) sugars. Entries shown in bold were conditions used experimentally. All of the entries shown in the table were used to simulate equilibrium sugar distributions.

<table>
<thead>
<tr>
<th>Case</th>
<th>Aliquat:N2B (mol/mol)</th>
<th>Sugar:N2B (mol/mol)</th>
<th>Aldose Sugar (M)</th>
<th>Aliquat 336 (M)</th>
<th>N2B (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5:1</td>
<td>0.67:1</td>
<td>0.110</td>
<td>0.248</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>1.5:1</td>
<td>1:1</td>
<td>0.165</td>
<td>0.248</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>1.5:1</td>
<td>1.5:1</td>
<td>0.248</td>
<td>0.248</td>
<td>0.165</td>
</tr>
<tr>
<td>2</td>
<td>2:1</td>
<td>0.67:1</td>
<td>0.110</td>
<td>0.330</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>1:1</td>
<td>0.165</td>
<td>0.330</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>1.5:1</td>
<td>0.248</td>
<td>0.330</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>2:1</td>
<td>0.330</td>
<td>0.330</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>2.5:1</td>
<td>0.413</td>
<td>0.330</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2.5:1</td>
<td>0.67:1</td>
<td>0.110</td>
<td>0.413</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2.5:1</td>
<td>1:1</td>
<td>0.165</td>
<td>0.413</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2.5:1</td>
<td>1.5:1</td>
<td>0.248</td>
<td>0.413</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2.5:1</td>
<td>2:1</td>
<td>0.330</td>
<td>0.413</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2.5:1</td>
<td>2.5:1</td>
<td>0.413</td>
<td>0.413</td>
<td>0.165</td>
</tr>
</tbody>
</table>

**4.2.1 SIRE**

The aqueous phase was pre-isomerized with 45g/L ofGX1 at 60°C for 5 hours. Data was collected every 50 min for the duration of the pre-isomerization. After 5 hours, the isomerization continued as described above but the aqueous media was placed into
contact with an organic media containing N2B and Q⁺Cl⁻. The SIRE process was carried out in a continuous fashion as shown in Figure 4-2. The aqueous phase was maintained at a constant pH of 8.5 and temperature of 60°C at the reservoir. The $GXI$ was contained in a large mesh bag and submersed in the aqueous phase reservoir to prevent intake into the pumping system. The organic phase was also heated to 60°C. The aqueous and organic phases were contacted across the hollow fiber membrane. The pumps were both balanced and calibrated to minimize the transfer of either phase across the hollow fiber membrane. Samples (~1.5 mL) were taken from both the aqueous and organic phases every 50 min for 5 hours. The aqueous layer was analyzed for sugar content using HPLC.

Figure 4-2: Schematic representation of SIRE-BE, illustrated for aldose isomerization to ketose. SIRE occurs at 60°C, the same temperature at which saccharified hydrolysate is available. The high affinity of the CA for ketose (xylulose and fructose) compared to aldose sugars (xylose and glucose) selectively extracts ketose into the organic phase, leaving behind a mostly aldose-rich solution. The $GXI$ was contained in a mesh bag within in the aqueous phase to prevent clogging of the pump and hollow fiber membrane.
4.2.2 Back-extraction

The organic phase samples were contacted with an equal volume of 0.5M HCl. The organic and aqueous back-extraction phases were allowed to mix for an hour at 60°C. The phases were then centrifuged at 5,000rpm for 5 min at room temperature and separated. The aqueous layer was analyzed for sugar content using HPLC.

4.2.3 Analytical methods

Aqueous samples collected from the experiments were diluted as needed and filtered through a 0.22 μm pore-size filter for HPLC. Standards for xylose, xylulose, glucose and fructose were used for HPLC calibrations. All aqueous samples and standards were analyzed using an Agilent 1100 HPLC system equipped with a refractive index detector (RID). Samples were analyzed using two Shodex SH1011 columns (300×8 mm, from Showa Denko K.K, Japan) in series. A mobile phase of 0.05 M H₂SO₄ was run at 0.6 ml/min; a column temperature of 50 °C and detector temperature of 35 °C were used for optimal peak resolution and detection. Chloride concentration was measured at room temperature using a Cole Parmer Combination Ion Selective Chloride Electrode. A chloride standard was used to calibrate the meter and correlate millivolts (mV) to chloride concentration.

4.2.4 Mathematical model development

4.2.4.1 SIRE model

The parameters for transient isomerization and RE parameters determined in Chapters 2 and 3 were used in a mathematical model created in MatLab for the simulation of the SIRE process (Appendix C). The isomerization equations as well as the
RE equations were used in this model. The equations for isomerization are shown in Equations 4.1-4.2.

\[
\frac{d|\text{Ketose}|}{dt} = -[E_c] \frac{V_m(|\text{Ketose}| - |\text{Ketose}|_e)}{K_m + (|\text{Ketose}| - |\text{Ketose}|_e)} \quad (4.1)
\]

\[
\frac{d|\text{Aldose}|}{dt} = -[E_c] \frac{V_m(|\text{Aldose}| - |\text{Aldose}|_e)}{K_m + (|\text{Aldose}| - |\text{Aldose}|_e)} \quad (4.2)
\]

Equations 4.1 and 4.2 were integrated in an ODESolver in MatLab. Simulations were conducted under conditions of dry enzyme addition to the aldose sugar solution; the empirical sugar loss term was included to account for non-specific sugar loss. The sugar loss terms (Eqn. 2.12 and 2.13) were calculated independently of the derivative and then subtracted from the aldose concentration calculated from the numerical integration of the derivative to update the aldose concentration.

The equilibrium constants for the reactive-extraction of sugars determined previously (Table 3.3) were used to predict the partitioning of each sugar between the aqueous and organic phases. Since the isomerization is slow relative to the reactive-extraction, the reactive-extraction was modeled as pseudo-steady state. The MatLab code was constructed as shown in Figure 4-3. The aqueous and organic phase volumes, amount of enzyme, [Aldose]o, [Aliquat® 336], [N2B], the experimental time, time of pre-isomerization and frequency of sampling were inputs for this model. The model simulates pre-isomerization for the pre-isomerization period, and then the model simulates reactive-extraction of the mixed aldose/ketose aqueous sugar solution. The model then loops through isomerization of the resulting aqueous phase sugar mixture for a period of time which is followed by reactive-extraction to redistribute sugars between the phases. The isomerization/reactive-extraction components continue to loop until the simulation reaches time equal to the experimental time input.
Figure 4-3. MatLab Simulation Schematic. Inputs for the concentration of N2B, Aliquat® 336, Aldose, pre-isomerization time, SIRE time, and sampling time are needed for the model simulation. The loop for isomerization/RE causes a sawtooth appearance to the aqueous simulation results. By reducing the isomerization time between RE calculations, the sawtooth appearance can be minimized. However, reducing the isomerization time substantially increases the total simulation time.

4.2.4.2 MatLab predictive simulations

MatLab simulations were run for varying N2B:Aliquat® 336 and Sugar:N2B ratios (see Table 4.1). The transient experimental results (sugar composition of aqueous and organic phases) were compared to several of these simulations (Table 4.1 bold entries). For all of the experiments and simulations, two calculations were made at the end of the experimental time period to compare the efficiency of the SIRE process under the specific experimental conditions.

Sugar extraction efficiency (SEE, Equation 4.3) is the ratio of total moles of sugar in the organic phase to the total initial moles of sugar in the aqueous phase (Equation
4.1. The SEE indicates how effective the SIRE process is at extracting sugar in general. The ketose extraction selectivity (KES, Equation 4.4) is the ratio of moles of ketose sugar in the organic phase to the total moles of sugar in the organic phase. The KES indicates how selective the organic phase is to ketose sugar extraction.

\[
\text{SEE} = \frac{\text{Total Sugar}_{\text{org}}}{\text{Initial Sugar}_{\text{aq}}} \quad (4.3)
\]

\[
\text{KES} = \frac{\text{Ketose}_{\text{org}}}{\text{Total Sugar}_{\text{org}}} \quad (4.4)
\]

4.2.4.3 Back-extraction model

The back-extraction was modeled with a modified reactive-extraction program (Appendix C). The final composition of the organic phase from SIRE is used as the input for this model. The reactive-extraction simulation was written for a system with a known equilibrium pH. While the initial pH of the aqueous phase is known for BE, the final pH depends on the sugar composition of the organic phase. Therefore, the back-extraction model was written to determine not only the sugar composition and chloride concentrations of each phase but also the final pH of the BE aqueous phase. Back-extraction experimental results were compared to the model predictions for sugar stripping under very low pH conditions. The back-extraction efficiency (BEE), defined in Equation 4.5, was calculated from the model to determine the efficiency of sugar extraction under the experimental stripping conditions.

\[
\text{BEE} = \frac{\text{Total Sugar Stripped}_{\text{aq}}}{\text{Total Sugar}_{\text{org}}} \quad (4.5)
\]

4.3 Results

The parameters determined from experimental data for the isomerization and reactive-extraction models and used for the comprehensive SIRE model accurately
predicts of the SIRE experimental data. Figure 4-4 shows the experimental results (data points) overlaid with the model predictions for Sugar:N2B:Aliquat® 336 ratios of 1:1:2.5 (Table 4.1, row 10). The results for SIRE experiments and model simulations are shown for glucose to fructose in Figure 4-4A and xylose to xylulose in Figure 4-4B. By including in the SIRE model the empirically-derived equation for sugar lost during isomerization, the model predictions are in excellent agreement with the experimental data. The total sugar lines (black) in Figure 4-4 are the MatLab predictions of the total sugar in both phases and mirrors that seen experimentally.

In looking at the equilibrium distribution of sugar between the two phases and isomeric forms, a clear trend is seen in the data, particularly in the sugar extraction efficiency (SEE) shown in Figure 4-5. As the ratio of initial sugar to N2B increases, the fraction of total sugar extracted (SEE) drops, although the total sugar extracted increases. As sugar to N2B increases, the ketose selectivity (KES) increases as shown in Figure 4-5. With increasing sugar:N2B, the competition for N2B binding sites increases, and more ketose is proportionately extracted.

The SEE seems to be independent of the N2B:Aliquat® 336 ratio for glucose while the KES increases slightly with increasing ratios of Aliquat® 336 to N2B. This is most likely due to the stability that the Aliquat® 336 imparts to the boronic acid-sugar complexes. Similar trends are seen for xylose to xylulose SIRE as shown in Figure 4-6. The SEE for both C5 and C6 sugars from the model predictions could be empirically modeled as a logarithmic decline; this empirical equation could be used to predict extraction under different conditions (see Figures 4-5 and 4-6).
Figure 4-4: Experimental data and model fits for SIRE mathematical model. (A) Glucose SIRE. (B) Xylose SIRE. Open symbols represent moles of organic sugar and closed symbols represent moles of aqueous sugar. The aqueous aldose solution was pre-isomerized for 5 hours. The organic media was introduced and SIRE continued for another 5 hours. The model simulation was extended past the experimental data collection until equilibrium was reached (~600 min after pre-isomerization). Data points represent the experimental data. Solid lines represent the mathematical model predictions under the same experimental conditions (Sugar:N2B:Aliquat® 336- 1:1:2.5). The black line shows the total soluble sugar in the system predicted by the model.
**Figure 4-5: KES and SEE for glucose SIRE.** Experimental data is shown as open data points. Closed data points represent the MatLab simulation results.

![Graph](image)

**Figure 4-6 KES and SEE for xylose SIRE.** Experimental data is shown as open data points. Closed data points represent the MatLab simulation results.

![Graph](image)

A more substantial difference is seen in Figure 4-6 for KES for SIRE with xylose. As the ratio of Aliquat® 336 increases, the reactive-extraction becomes more selective for ketose isomers, thereby increasing the KES with increasing ratios of Sugar:N2B.
Figure 4-7 shows the model predictions of the various CA species predicted at the end of the SIRE experiment. Much of the CA is found ion-paired with Q$^+$ for all simulated conditions as evidenced by the lightest colored bars. With increasing ratios of Aliquat®336:N2B, the percentage of CA bound to Q$^+$ increases. Additionally, with higher

![Graph A](image1)

![Graph B](image2)

**Figure 4-7: Model Predictions of %CA$_{species}$ at the end of SIRE simulations.** All simulations used parameters described in Table 4.1. Panel A shows the model predictions of the % CA in each species for glucose SIRE after 300 min pre-isomerization and 300 min SIRE. Panel B shows the model predictions of the % CA in each species for xylose SIRE after 300 min pre-isomerization and 300 min SIRE.
ratios of Aliquat®336:N2B and sugar:N2B, the amount of ion-paired CA bound to sugar increases.

To measure sugar concentrations in the organic phase, the organic phase sugar was back-extracted into a very low pH stripping phase. Approximately 97% of sugar was calculated as successfully recovered from the organic phase (BEE, Equation 4.3). The slight overestimate of the model for the organic phase sugar composition during SIRE can be attributed to the ~3% of the sugar not recovered during BE. The BE mathematical model predicts comparable sugar concentrations to those obtained experimentally with very low pH stripping solutions.

4.4 Discussion

The comprehensive SIRE-BE model developed closely predicts the data for SIRE-BE collected experimentally. Additionally, by calculating the SEE and KES, a quick assessment can be made to fine tune ratios of sugar, N2B and Aliquat® 336 to optimize ketose recovery and purity during SIRE. The transient SIRE model can be used to determine the effects of varying enzyme concentration and sugar concentrations as well as organic phase components. Figures 4-5 and 4-6 show a clear trade-off between overall sugar extraction efficiency and ketose selectivity.

By increasing the ratio of sugar to N2B, the total sugar extracted and the ketose extraction efficiency (KES) increase but with lower sugar extraction efficiency (SEE) from the aqueous phase. Figure 4-7 shows that the model predicts that the vast majority of CA is ion-paired with the cation (Q⁺) of Aliquat®336. Additionally, increasing the amount of Aliquat® 336, and therefore chloride, in the organic phase allows for greater ketose selectivity, although this trend levels off at higher ratios of sugar:N2B.
Aldose and ketose sugars can be separated from one another during back-extraction BE by employing a two-stage stripping procedure. For Stage 1, the organic phase is placed into contact with a mildly acidic aqueous phase (pH 5-6) to extract weakly-complexed aldose sugars. The sugar remaining in the organic phase can then be recovered in a Stage 2 with a lower pH (1-2) aqueous phase stripping solution. This staged stripping approach applied to RE of xylose and xylulose has been shown to yield a nearly pure xylulose sugar stream that may be used to produce a variety of biobased products. The mathematical model of BE could be used to predict the expected sugar released to the aqueous phase and remaining in the organic phase following the Stage 1 and Stage 2 stripping under different pH conditions.

The resulting ketose sugar stream generated from back-extraction can be used for downstream applications such as dehydration of sugars into furans. The back-extraction process leaves a concentrated source of relatively pure ketose sugars dissolved in acidic media. Dehydration is catalyzed by acid such as the HCl used to back-extract the sugars. The following chapter addresses the modeling of the dehydration of the ketose sugar xylulose to furfural.
Chapter 5

Xylulose dehydration to furfural

5.1 Introduction

Furfural is projected to be in high demand with its increasing prevalence in areas such as petroleum refining, plastics, agrochemical and pharmaceutical industries.\textsuperscript{38} It is very versatile and is a key platform chemical for the synthesis of many useful products and fuels, including 2-methylfuran, gasoline and diesel components.\textsuperscript{39} The current industrial production of furfural occurs through acid treatment of the hemicellulosic fraction of lignocellulosic biomass. A Brønsted acid is used to depolymerize xylan to xylose and subsequently catalyzes the dehydration of xylose to furfural.\textsuperscript{40-42} With a process yield of 30-50\%, this method is severely hampered by the side-reactions such as humin formation, which consume the sugar as well as furfural.\textsuperscript{41,43} It is well-established that the activation energy for humin formation is lower than for the dehydration reaction.\textsuperscript{44} To reduce the effect of humin formation and maximize overall furfural production, the dehydration of xylose to furfural is conducted at low xylose concentrations and at high temperatures (170-185 °C).
Brønsted acid-catalyzed xylose dehydration to furfural appears to occur through a direct cyclic mechanism via a furan aldehyde intermediate.\textsuperscript{47-49} Another strategy utilized is to isomerize the xylose to its ketose isomer, xylulose, via Lewis acid catalysts\textsuperscript{46}. Xylulose dehydration has also been used to produce furans.\textsuperscript{38, 45, 49-52} The dehydration of ketose sugars to furans has a lower activation energy compared to its aldose counterparts. In theory, this allows the dehydration reaction to be performed at higher initial sugar concentrations and with lower temperatures to achieve a reduction in humin formation and thereby a higher yields of furan.\textsuperscript{8, 38, 45, 49-53} However, the isomerization-dehydration route is hampered by the unfavorable equilibrium of the isomerization which favors the aldose sugar. In order to drive the isomerization reaction toward ketose production, product removal strategies that combined the isomerization reaction and dehydration reaction have been attempted.\textsuperscript{45, 49, 51, 52, 54} At temperatures greater than 140 °C, a combination of Lewis and Brønsted acid catalysts have been used to increase furan yields.\textsuperscript{45, 49, 54} However, these mixed acid catalyst reactions for the conversion of xylose to furfural show that the presence of the Lewis acid promotes not only the desired sugar isomerization, but also the formation sugar and furfural degradation products, while the Brønsted acid promotes xylulose to furfural conversion.\textsuperscript{38, 45} Due to the limitations of this combined acid catalyst system, the proportion of Lewis to Brønsted acid is critical for maximizing furfural yield through the ketose intermediate.\textsuperscript{55}

In light of these limitations, a novel approach for high-yield furfural production via a ketose intermediate (xylulose) has been reported.\textsuperscript{20} This method uses a staged approach and is capable of producing high furfural yields while mitigating the limitations that commonly hinder the success of the mixed acid catalyst process. First the aldose
sugar undergoes simultaneous-isomerization with reactive-extraction of the resulting ketose sugar to an immiscible organic phase. Then the sugar-rich organic phase is back-extracted with acidified water to produce a concentrated acidic sugar solution. The subsequent acidic sugar solution is then heated at lower temperatures (<130°C) to dehydrate the ketose to furfural. While a number of kinetic studies and mathematical models have been developed to understand the reaction pathways of the dehydration of xylose to furfural\textsuperscript{44.56-59}, no models exist to capture the process of furfural production starting from xylulose.

In this chapter, a mathematical model is developed and tested for its ability to predict transient dehydration of xylulose to furfural. The model includes the temperature dependence associated with the reaction rate constants.

**5.2 Methods and materials**

**5.2.1 Experimental set-up**

The experimental data in this section were collected by Bin Li as a part of his dissertation and have been published\textsuperscript{37}.

**5.2.2 SIRE-BE**

High purity, concentrated xylulose was produced from xylose by simultaneous-isomerization-and-reactive-extraction (SIRE) followed by a two-stage back-extraction (BE). Briefly\textsuperscript{37}, SIRE was conducted using 40mM xylose in 50 mM sodium phosphate buffer at pH 8.5 and 50 °C with 4.5 g/l Gensweet\textsuperscript{®} IGI (immobilized xylose isomerase); the aqueous sugar mixture was contacted with an equal volume of organic phase (octanol containing 132 mM naphthalene-2-boronic acid (N2B) and 330 mM Aliquat\textsuperscript{®} 336). The sugars extracted into the organic phase were back-extracted into HCl-acidified water in
two stages using a reduced stripping phase volume to concentrate the sugars and to increase the xylulose purity. The first stage stripping removed the majority of the xylose and resulted in a slight loss of xylulose. The second stage stripping extracted concentrated xylulose at ~220mM with a small amount of xylose (~4mM).

5.2.3 Furfural production from xylulose

The dehydration experiments were carried out in well-mixed 10 ml thick-walled glass vials. A 1 ml sample of the xylulose (220mM, pH 1) solution in water was added to the reaction vial with a magnetic stir bar and the vessel was sealed (Case 1). Experiments were also conducted with reaction media that consisted of a mixture of water and dimethyl sulfoxide (DMSO) (Case 2). For in situ product extraction experiments from aqueous reaction media, 1 mL of an immiscible organic solvent was also added to the reaction vial (Case 3). A summary of the experimental cases is shown in Table 5.1. Xylulose was dehydrated at temperatures ranging from 100-130 °C. In all cases, the vials were heated in an oil bath on top of a stirring hotplate. Multiple vials were started simultaneously with each being removed after a different reaction time. Vials were quenched in an ice bath immediately upon removal from the heated oil bath. Transient data was collected and analyzed for xylose, xylulose and furfural concentrations.

Table 5.1: Summary of Experimental Cases. All cases started with ~220mM xylulose at pH 1.

<table>
<thead>
<tr>
<th>Case</th>
<th>Aqueous Composition</th>
<th>Organic Extraction Phase to Aqueous Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H₂O</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>2:1 (v/v) H₂O:DMSO</td>
<td>-</td>
</tr>
<tr>
<td>2b</td>
<td>1:2 (v/v) H₂O:DMSO</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>H₂O</td>
<td>1:1 (v/v)</td>
</tr>
</tbody>
</table>
5.2.4 Analytical methods

Aqueous and organic phase samples collected from the experiments were diluted as needed and filtered through a 0.22 μm pore-size filter for HPLC and GC analysis. Standards for xylose, xylulose and furfural were used for HPLC calibrations. All aqueous samples and standards were analyzed using an Agilent 1100 HPLC system equipped with a refractive index detector (RID). Samples were analyzed using two Shodex SH1011 columns (300×8 mm, from Showa Denko K.K, Japan) in series. A mobile phase of 0.05 M H₂SO₄ was run at 0.6 ml/min; a column temperature of 50 °C and detector temperature of 35 °C were used for optimal peak resolution and detection. Furfural in the organic phase samples was quantified via gas chromatography using a Shimadzu 2010 gas chromatograph equipped with a Restek (Bellefonte, PA) Rtx Bio-diesel column (15 m x 0.32 mm ID x 0.1 Om) and a flame ionization detector (FID). The GC oven temperature program was held at 40 °C for 4 min and then raised to 100 °C at a ramp rate of 5 °C/min and from 100 to 250 °C at a ramp rate of 20 °C/min. Helium was used as a carrier gas with a constant linear velocity of 50 cm/s in the column. The injector temperature and FID temperature were both maintained at 250 °C.

5.2.5 Computational model

While dehydration of xylose to furfural has been experimentally measured and theoretically modeled,⁴⁴ dehydration of xylulose to furfural has been only recently reported. Side-reactions considered important during xylose dehydration include first-order loss of furfural to by-product as well as second-order cross-condensation of xylose and furfural.⁴⁴ However, it is unknown if similar mechanisms of reactant and product loss are likely to be important for xylulose dehydration, especially considering that xylulose
dehydration occurs under considerably lower temperatures than is possible for xylose. In the reaction models evaluated here, mechanisms of xylulose and furfural loss illustrated in Figure 5-1 were assessed by comparing the predictions of the computational models to the transient xylulose and furfural measurements. The model shown in Figure 5-1A was developed for the single-phase dehydration (Cases 1 & 2) while Figure 5-1B includes *in situ* extraction of furfural to an immiscible organic phase (Case 3). Parallel reactions of residual xylose present in the sugar solution were neglected due to xylose's low concentration in the sugar solution but also due to its low reactivity under the experimental conditions used for xylulose dehydration.

![Figure 5-1: Proposed reaction pathways for xylulose dehydration to furfural.](image)

**(A)** Single-phase dehydration. **(B)** Biphasic dehydration with aqueous phase dehydration and *in situ* furfural extraction to an organic phase.

The reaction models illustrated in Figure 5-1 were translated into unsteady-state mass balances for xylulose and furfural assuming mass-action kinetics with unknown reaction rate constants. Reactions within a homogenous single phase (Figure 5-1A) for xylulose (Y) and furfural (F) are given by Equations 5.1 & 5.2, respectively.

\[
\frac{dy}{dt} = -k_1 Y - k_2 Y - k_3 Y^2 - k_4 YF
\]  

(5.1)
Dehydration coupled with \textit{in situ} furfural extraction into an immiscible organic phase was modeled by including reversible transfer of furfural between the two phases as shown in Equations 5.4 and 5.5 as the $k_7$ and $k_8$ terms:

\[
\frac{dF}{dt} = k_1Y - k_4YF - k_5F - k_6F^2 
\]

\[
\frac{dY}{dt} = -k_1Y - k_2Y - k_3Y^2 - k_4YF_{aq} 
\]

\[
\frac{dF_{aq}}{dt} = k_1Y - k_4YF_{aq} - k_5F_{aq} - k_6F_{aq}^2 - k_7F_{aq} + k_8F_{org} 
\]

\[
\frac{dF_{org}}{dt} = k_7F_{aq} - k_8F_{org} 
\]

The mathematical models were solved in MatLab (see Appendix D). For data sets at multiple temperatures, the transient data was fit as a complete set assuming the rate constants at each temperature obeyed an Arrhenius dependency. Based on initial estimates, optimization of each reaction pathway’s activation energy ($E_a$) and pre-exponential factor ($A$) was achieved by using a genetic algorithm. The genetic algorithm, constrained with bounds to ensure positive $E_a$ and $A$, obtained a global minimum for the coupled ODEs over the entire temperature range for a given reaction media. The algorithm randomly selected values within the bounded range and, in a process that mimics biological evolution, adapted each subsequent value to obtain an optimal solution. The sum-squared error ($E$) between the experimental (subscripted $e$) and model (subscripted $m$) values for both xylulose and furfural shown in Equation 5.6 was calculated for each iteration.

\[
E = \sum_{T=1}^{z} \sum_{t=1}^{x} (F_{m,T} - F_{e,T})^2 + (Y_{m,T} - Y_{e,T})^2 
\]

In this equation, the inner summation is over $x$ time points at one temperature and the outer summation is over $z$ temperatures. A simulation ended when the difference in
the sum-squared error between sequential iterations fell below the pre-established tolerance ($10^{-9}$). Due to the number of parameters, the error minimization was conducted 10 times for each data set to ensure that the model converged reproducibly. Using the best-fit values for $E_a$ and $A$ from each minimization run, the reaction rate constants ($k_1$-$k_6$) were calculated for each temperature. The average of each rate constant at each temperature was calculated from the 10 minimization runs. The contribution of each reaction pathway to xylulose consumption under different reaction conditions was determined by comparing the fraction of xylulose passing through each competing pathway at any time during the reaction.

The experimental data collected with *in situ* furfural extraction to an immiscible organic phase was evaluated using the reaction pathways shown in Figure 5-1B and Equations 5.3-5.5. Rate constants ($k_1$-$k_6$) established from analysis of Case 1 data were fixed for analysis of the Case 3 data. The reaction rate constants associated with reversible furfural transfer from the aqueous layer to the organic layer ($k_7$ and $k_8$) were the only parameters fit for the Case 3 data set. The method employed for parameter estimation of $k_7$ and $k_8$ mirrored that described for calculating $k_1$-$k_6$ from the single-phase data.

**5.3 Results and discussion**

The results of the computational model analysis for each experimental case are discussed in the following subsections.

5.3.1 Case 1: Dehydration of xylulose in water

The model fits to the experimental data for both xylulose and furfural in aqueous media are shown in Figure 5-2. The model predictions are in close agreement with the
xylulose data (Figure 5-2A) for all temperatures, capturing the increased rate of xylulose consumption seen experimentally at higher temperatures. Model predictions for furfural production (Figure 5-2B) are also in good agreement with the experimental data, showing both the rapid production of furfural via dehydration of xylulose as well as its loss by side reactions. The model accurately describes the timing and magnitude of the furfural maximum that occurs at each temperature. In an industrial setting, the reactions would be stopped at the furfural maximum to minimize degradation of the furfural product.

**Figure 5-2: Model fits to xylulose dehydration data in aqueous reaction media.** The experimental data were generated with xylulose at an initial concentration of ~220mM. Closed symbols (A) denote xylulose and open symbols (B) denote furfural. Experimental data are the average of duplicate runs; error bars are standard deviation. Solid lines show the model predictions of xylulose consumption and furfural accumulation using the experimental conditions.

5.3.2 Case 2: Dehydration of xylulose in water/DMSO

At elevated temperatures under acidic conditions, water molecules have been shown to promote undesirable cross-polymerization reactions between the furan-product and the sugar-reactant. For this reason, aprotic solvents such as dimethyl sulfoxide (DMSO) have been used to enhance product yield by lowering or eliminating the sugar-
and/or furan–water interactions.\textsuperscript{44} The experimental data shows that DMSO improves the outcome of xylulose dehydration to furfural, with higher DMSO concentrations resulting in faster furfural production and slower furfural loss.

In fitting the model parameters to these experimental data, each DMSO concentration was fit independently. As shown in Figure 5-3, the model fits have excellent agreement with the experimental data for both concentrations of DMSO. For the highest temperature dehydrations (130°C), the models predict faster xylulose consumption and furfural production at short times than was measured experimentally. However, this is likely an artifact of the experimental data collection as the thick-walled glass vials used for these kinetic experiments may not be strictly isothermal for such short reaction times. Thus, the experimental data likely underestimates the isothermal xylulose consumption and furfural production for very short times (< 6 min).

Figure 5-4 shows model predictions for xylulose consumed by each pathway at 100°C. Dehydration of xylulose (k\textsubscript{1}) accelerates in the presence of DMSO. Cross-condensation of xylulose and furfural (k\textsubscript{4}) appears to be the dominate side-reaction for xylulose loss in all cases. In the presence of DMSO, the model predicts a significant decrease in first and second order loss products (k\textsubscript{2} and k\textsubscript{3}). At the highest DMSO concentration (Figure 5-4C), cross-condensation (k\textsubscript{4}) is also significantly reduced.

A similar analysis of model predictions is shown for furfural at 100°C (Figure 5-5) where both the furfural produced by xylulose dehydration (k\textsubscript{1}) and loss pathways (k\textsubscript{4}-k\textsubscript{6}) are shown. The model predicts that the dominate loss of furfural is due to the cross-condensation of xylulose and furfural (k\textsubscript{4}) followed by second-order resinification of
furfural \((k_6)\) and first-order furfural loss \((k_5)\). When DMSO is added to the reaction media, loss of furfural to cross-condensation products \((k_4)\) is reduced.

**Figure 5-3: Model fits to experimental data collected in H₂O/DMSO reaction media.**

Experimental data shown are for xylulose dehydration in two different compositions of the reaction phase: \((A,B) 2:1 \text{ (v/v) } \text{H}_2\text{O}:\text{DMSO} \text{ and (C,D) 1:2 \text{ (v/v) } \text{H}_2\text{O}:\text{DMSO}.} \)

Xylulose consumption is shown in \((A,C)\) with furfural accumulation shown in the adjacent panels \((B,D)\). Closed symbols denote xylulose and open symbols denote furfural. Experimental data are the average of duplicate runs; error bars are standard deviation. Solid lines show the model predictions for the same starting conditions as used experimentally.
Figure 5-4: Model predictions of the percentage of xylulose consumed passing through each pathway at 100°C. (A) Water (0% DMSO). (B) 2:1 (vol/vol) H$_2$O:DMSO. (C) 1:2 (vol/vol) H$_2$O:DMSO. The xylulose reactions shown are: $k_1$ – dehydration to furfural; $k_2$ – first-order loss to side-products; $k_3$ – second-order loss to side-products; and $k_4$ – cross-condensation of xylulose with furfural.
Figure 5-5: Percentage of furfural through each pathway at 100°C. (A) 0% DMSO media. (B) 2:1 (vol/vol) H₂O:DMSO. (C) 1:2 (vol/vol) H₂O:DMSO. The xylulose reactions shown are: k₁ – dehydration to furfural; k₄ – cross-condensation of xylulose with furfural; k₅ – first-order loss to side-products; k₆ – second-order loss to side-products.

To further analyze the reactions associated with xylulose dehydration, the model predictions of activation energies (Eₘ) and pre-exponential factors (shown as log₁₀A) for each reaction pathway are summarized in Table 5.2. For the dehydration of xylose to furfural in aqueous media, the activation energy has been reported to be 123.91±6.00 kJ/mol.⁴⁴ Xylulose dehydration to furfural (k₁) in aqueous media is predicted to be 83.39±1.75 kJ/mol. The reduced activation energy allows xylulose dehydration to take
place at lower temperatures than xylose (>140 °C) and may be responsible for the increased furfural yield compared to that reported for xylose (67% for xylulose at 100 °C, ~12% for xylose at 140 °C). 

Table 5.2: Estimated activation energies and pre-exponential factors for monophasic systems.  

<table>
<thead>
<tr>
<th>Reaction phase</th>
<th>Arrhenius constants</th>
<th>$k_1$ (1/hr)</th>
<th>$k_2$ (1/hr)</th>
<th>$k_3$ (1/hr·mM)</th>
<th>$k_4$ (1/hr·mM)</th>
<th>$k_5$ (1/hr)</th>
<th>$k_6$ (1/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>$E_a$ (kJ/mol)</td>
<td>83.39±1.75</td>
<td>125.22±2.39</td>
<td>92.12±2.88</td>
<td>75.22±1.91</td>
<td>88.21±2.45</td>
<td>64.31±2.47</td>
</tr>
<tr>
<td></td>
<td>$\log_{10}A$</td>
<td>11.68±0.24</td>
<td>16.01±0.30</td>
<td>8.74±0.29</td>
<td>7.70±0.31</td>
<td>7.38±0.38</td>
<td>5.53±0.34</td>
</tr>
<tr>
<td>Water: DMSO 2:1 (v/v)</td>
<td>$E_a$ (kJ/mol)</td>
<td>137.07±1.95</td>
<td>102.94±5.14</td>
<td>364.40±3.53</td>
<td>146.64±1.88</td>
<td>233.12±4.03</td>
<td>53.95±2.76</td>
</tr>
<tr>
<td></td>
<td>$\log_{10}A$</td>
<td>19.48±0.27</td>
<td>12.73±0.29</td>
<td>44.44±0.19</td>
<td>17.83±0.21</td>
<td>27.70±0.21</td>
<td>3.71±0.35</td>
</tr>
<tr>
<td>Water: DMSO 1:2 (v/v)</td>
<td>$E_a$ (kJ/mol)</td>
<td>131.30±2.56</td>
<td>102.10±5.29</td>
<td>360.91±6.01</td>
<td>142.24±2.45</td>
<td>232.67±3.15</td>
<td>52.98±1.69</td>
</tr>
<tr>
<td></td>
<td>$\log_{10}A$</td>
<td>19.33±0.36</td>
<td>12.74±0.32</td>
<td>44.39±0.27</td>
<td>17.68±0.32</td>
<td>27.76±0.19</td>
<td>3.67±0.23</td>
</tr>
</tbody>
</table>

* Standard deviation from 10 simulations for parameter estimations

The activation energy predicted by the model for the second-order reaction of xylulose and furfural ($k_4$, 75.22±1.91kJ/mol) in the aqueous reaction media is actually lower than that predicted for xylulose dehydration ($k_1$). The lower $E_a$ coupled with a large pre-exponential factor allows the cross-condensation of xylulose and furfural to happen readily at low dehydration temperatures once furfural begins to accumulate. The addition of DMSO increases the activation energy required for the xylulose dehydration reaction but also increases the energy required for the xylulose-furfural cross-condensation reaction. Table 5.3 shows the reaction rate parameters for 100°C for Case 1, 2a and 2b. A marked increase in the dehydration rate constant ($k_1$) is noted with increasing amounts of DMSO while a sharp decrease in the rate constant for second-order loss of xylulose ($k_3$) is seen.
Table 5.3: Estimated reaction rate parameters for 100°C.

<table>
<thead>
<tr>
<th>Reaction phase</th>
<th>k₁ (1/hr)</th>
<th>k₂ (1/hr)</th>
<th>k₃ (1/hr·mM)</th>
<th>k₄ (1/hr·mM)</th>
<th>k₅ (1/hr)</th>
<th>k₆ (1/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>8.45E-01</td>
<td>3.00E-02</td>
<td>6.87E-05</td>
<td>1.45E-03</td>
<td>1.06E-05</td>
<td>3.32E-04</td>
</tr>
<tr>
<td>Water:DMSO 2:1 (v/v)</td>
<td>1.91E+00</td>
<td>2.08E-02</td>
<td>2.58E-07</td>
<td>1.97E-03</td>
<td>1.12E-05</td>
<td>1.43E-04</td>
</tr>
<tr>
<td>Water:DMSO 1:2 (v/v)</td>
<td>8.84E+00</td>
<td>2.75E-02</td>
<td>7.11E-07</td>
<td>5.80E-03</td>
<td>1.52E-05</td>
<td>1.77E-04</td>
</tr>
</tbody>
</table>

5.3.3 Case 3: *In situ* product extraction from an aqueous reaction mixture

*In situ* product removal from the aqueous reaction medium to an immiscible extraction solvent was the last set of xylulose dehydration data that was modeled (see Figure 5-1B). Rapid extraction of furfural from the aqueous reaction medium reduces the concentration of furfural in the reaction phase, mitigating the impact of side-reactions that lower furfural yield. Partitioning of furfural into the organic phase was modeled as a reversible reaction, and the forward and reverse rate constants for this transfer were fit to the experimental data. Rate constants associated with all other aqueous phase reactions were assumed unaffected by furfural extraction into the organic phase and were held fixed at the best-fit values determined in the Case 1 simulations at the same temperature. The model predictions and the experimental data are shown in Figure 5-6. Furfural extraction was found to improve furfural yields and minimize unwanted side reactions. Interestingly, the kinetic profiles for the consumption of xylulose are dependent on the organic phase.

The partition coefficient for furfural between the reaction phases, $F_{org}/F_{aq}$, is summarized in Table 5.4 for both experimental and model predictions. Within the model framework, $\frac{dF_{org}}{dt}$ (Equation 5.5) can be assumed to be pseudo steady-state due to the magnitude of $k_7$ and $k_8$ so the partition coefficient can be expressed as $k_7/k_8$. The
magnitude of the model-predicted values for \(k_7\) and \(k_8\) supports a pseudo-steady state partitioning assumption. Differences in furfural yield or time-to-maximum yield summarized in Table 5.4 do not correlate to the furfural partition coefficient. As shown in Table 5.4, the experimentally-measured and model-predicted partition coefficients are similar.

**Figure 5-6: Model fits to experimental data collected in biphasic reaction media.** Experimental data shown are for xylulose dehydration in four different biphasic reaction media as noted in each pane. Experimental data are the average of duplicate runs; error bars are standard deviation. Solid lines show the model predictions for the same starting conditions as used experimentally.
Table 5.4: Summary of maximum measured furfural production and xylulose consumption from biphasic xylulose dehydration experiments at 110°C. Total furfural yield is based on furfural in both the organic and aqueous phases.  

<table>
<thead>
<tr>
<th>Organic phase</th>
<th>Experimental</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (hrs)</td>
<td>Xylulose consumption (%)</td>
</tr>
<tr>
<td>Toluene</td>
<td>3</td>
<td>99</td>
</tr>
<tr>
<td>MIBK</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td>2-sec butyl phenol</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>2-butanol/MIBK (7:3 v/v)</td>
<td>3</td>
<td>100</td>
</tr>
</tbody>
</table>

5.3.4 Simplified Model

Based on the results of Case 1, 2a and 2b, the model was simplified as shown in Equations 5.7 and 5.8 to include only the major contributors to xylulose and furfural loss. The mathematical models were solved in the same way as the expanded model. Table 5.5 shows the estimated activation energies and pre-exponential factors for this simplified analysis. These values are close to the values shown in Table 5.2 for the expanded model, indicating that the simplified model can be used as an accurate description of these three experimental cases.

\[
\frac{dY}{dt} = -k_1 Y - k_4 Y F \tag{5.7}
\]

\[
\frac{dF}{dt} = k_1 Y - k_4 Y F - k_6 F^2 \tag{5.8}
\]

The model fits to the experimental data for both xylulose and furfural in aqueous media are shown in Figure 5-7. The model predictions are in close agreement with the xylulose data (Figure 5-7A) for all temperature, capturing the increased rate of xylulose consumption seen experimentally at increasing temperatures. Model predictions for furfural production (Figure 5-7B) are also in good agreement with the experimental data, showing both the rapid production of furfural via dehydration of xylulose as well as its
loss by side reactions. The simplified model accurately describes the timing and magnitude of the furfural maximum that occurs at each temperature.

Table 5.5: Estimated activation energies and pre-exponential factors for simplified monophasic systems.

<table>
<thead>
<tr>
<th>Reaction phase</th>
<th>Arrhenius constants</th>
<th>Simplified Model</th>
<th>Original Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_a$ (kJ/mol)</td>
<td>$k_1$ (1/hr)</td>
<td>$k_2$ (1/hr·mM)</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>84.31±1.41</td>
<td>74.60±2.36</td>
</tr>
<tr>
<td></td>
<td>log$_{10}A$</td>
<td>11.67±0.21</td>
<td>7.58±0.31</td>
</tr>
<tr>
<td>Water: DMSO 2:1 (v/v)</td>
<td>$E_a$ (kJ/mol)</td>
<td>138.02±0.66</td>
<td>146.58±1.88</td>
</tr>
<tr>
<td></td>
<td>log$_{10}A$</td>
<td>19.55±0.09</td>
<td>17.84±0.14</td>
</tr>
<tr>
<td>Water: DMSO 1:2 (v/v)</td>
<td>$E_a$ (kJ/mol)</td>
<td>136.25±2.46</td>
<td>145.08±2.48</td>
</tr>
<tr>
<td></td>
<td>log$_{10}A$</td>
<td>19.48±0.13</td>
<td>17.76±0.20</td>
</tr>
</tbody>
</table>

*Standard deviation from 10 simulations for parameter estimations

Figure 5-7: Simplified model fits to xylulose dehydration data in aqueous reaction media. The experimental data were generated with xylulose at an initial concentration of ~220mM. Closed symbols (A) denote xylulose and open symbols (B) denote furfural. Experimental data are the average of duplicate runs; error bars are standard deviation. Solid lines show the simplified model fits of xylulose consumption and furfural accumulation using experimental conditions.

As shown in Figure 5-8, the simplified model fits have excellent agreement with both concentrations of DMSO used experimentally. Therefore, although the other two
pathways may be present, they are such minor contributors to the reaction pathways that the predictive ability of the simplified model is equivalent to the more complex model.

![Graphs showing xylulose and furfural accumulation over time at different temperatures and solvent compositions.](image)

**Figure 5-8: Simplified model fits to experimental data collected in H₂O/DMSO reaction media.** Experimental data shown are for xylulose dehydration in two different compositions of the reaction phase: (A,B) 2:1 (v/v) H₂O:DMSO and (C,D) 1:2 (v/v) H₂O:DMSO. Xylulose consumption is shown in (A,C) with furfural accumulation shown in the adjacent panels (B,D). Closed symbols denote xylulose and open symbols denote furfural. Experimental data are the average of duplicate runs; error bars are standard deviation. Solid lines show the model predictions for the same starting conditions as used experimentally.

### 5.4 Conclusions

Both of the models developed show excellent agreement with the experimental data for all temperatures and solvent compositions tested. The model parameters for dehydration in DMSO media were more challenging to determine due to the rapid rate of
furfural production (higher experimental error in the data) and the stiffness of the equations.

For reaction media containing DMSO, the dehydration reaction rate constant significantly increased relative to water at the same temperature. The loss of xylulose and/or furfural associated with side-reactions decrease with the addition of DMSO to the aqueous reaction phase. Aprotic solvents or in situ removal of furfural from the aqueous layer by an immiscible solvent is necessary to protect the furfural from detrimental side-reactions (namely $k_4$ and $k_6$). Allowing the reaction to proceed past the time when the maximum furfural yield is reached gives rise to unnecessary product losses ($k_5$ and $k_6$). These furfural losses can be mitigated by simply terminating the reaction as soon as the maximum furfural yield is reached or by reducing the reaction temperature.

The reduction in reaction pathways in the model to only the dehydration of xylulose to furfural ($k_1$) and the two major reactions leading to both reactant and product loss ($k_4$ and $k_6$) resulted in accurate predictions for both the xylulose and furfural time-courses. This model simplification allowed the genetic algorithm in the MatLab program to solve for fewer unknowns (6 unknowns compared to 14 used in the original expanded model), thereby decreasing simulation time.
Chapter 6

Future Work

As demand increases for oil and chemicals derived from fossil fuels, alternative sources of renewable energy will be needed to meet the demand. Optimizing and developing mathematical models for known and effective processes allow these critical products to be made in a cost-effective and efficient manner.

The overall SIRE model is tuned for and very accurate at predicting the results of sugar isomerization with reactive-extraction at 60°C and pH 8.5. The streamlined laboratory experimental protocol outlined for enzyme kinetics allows for expansion to varied temperature and pH isomerization conditions to create a robust kinetic model for biomass sugars conversion. The experiments described in Chapter 3 for determining the reactive-extraction parameters can be used as a framework to screen different complexing agents and lipophilic salts or alternative organic solvents. The results of the 3 sets of experiments outlined in Table 3.1 can be conducted in the laboratory quickly, with minimal input of time and reagent resources. The resulting equilibrium parameters can be plugged into the MatLab model to simulate optimal operating conditions for SIRE without expending a large amount of time and resources in the lab. The SIRE model
could also be expanded to include mixed-sugar SIRE predictions to more closely simulate the conditions faced in an industrial/production setting.

Nearly all of the sugar can be back-extracted to an acidic aqueous phase by using a large amount of hydrochloric acid to regenerate the Aliquat. A two-stage BE model could be developed to predict the separation of aldose/ketose sugars from the organic phase following SIRE and validated experimentally.

Finally, the relatively pure and concentrated ketose sugar streams can be used to produce numerous valuable products, such as furans which are precursors for drop in fuels and plastics. By understanding the reaction kinetics for xylulose dehydration into furfural, the conversion of the valuable sugar feedstock can be optimized and made predictable.
References

1. NREL, *What Is a Biorefinery?*


25. Li, B., Relue, P., and Varanasi S. Reactive extraction of biomass sugars for more efficient downstream conversion to products. in *AIChe Annual Meeting*. 2011. Minneapolis, MN.


Appendix A

Enzyme Kinetic MatLab Code

function [estimates, ssetotal] = ekineticsfit(x,y);

%x1=time in min
x1 = [
];
%enter first set of concentrations aldose and ketose
y1 = [
];
%x2=time in min
x2=[
];
%enter second set of concentrations aldose and ketose
y2=[
];
%x3=time in min
x3=[
];
%enter third set of concentrations aldose and ketose
y3=[
];

%enter amount enzyme in g/L
Et=;

format short eng

tic
model = @ode;
options=gaoptimset('Generations', 3000, 'TolFun', 1e-9, 'TolCon', 1e-8);
%enter lower bounds for Vmf Vmr kmf kmr
lb=[];
% enter upper bounds for Vmf Vmr kmf kmr
ub = []; 

[estimates, ssetotal, exitflag, output] = ga(model, 4, [], [], [], [], lb, ub, [], options)

function [ssetotal] = ode(params)
    p(1) = params(1);       p(2) = params(2);
    p(3) = params(3);       p(4) = params(4);

    [t1ode, y1ode] = ode15s(@(t,y)odefileEK1(t,y,p), x1, y1(1,:));
    [t2ode, y2ode] = ode15s(@(t,y)odefileEK2(t,y,p), x2, y2(1,:));
    [t3ode, y3ode] = ode15s(@(t,y)odefileEK3(t,y,p), x3, y3(1,:));

    FittedCurve1 = y1ode;
    ErrorVector1 = (y1 - FittedCurve1);
    sse1 = sum(sum(ErrorVector1.^2));

    FittedCurve2 = y2ode;
    ErrorVector2 = (y2 - FittedCurve2);
    sse2 = sum(sum(ErrorVector2.^2));

    FittedCurve3 = y3ode;
    ErrorVector3 = (y3 - FittedCurve3);
    sse3 = sum(sum(ErrorVector3.^2));
    ssetotal = sse1 + sse2 + sse3;

end

figure(1)
plot(x1, y1, 'x')
hold on
plot(x1, y1ode, '-')
hold off
axis([0 x1(end) 0 y1(1,1)])
figure(2)
plot(x2, y2, 'x')
hold on
plot(x2, y2ode, '-')
hold off
axis([0 x2(end) 0 y2(1,1)])
figure(3)
plot(x3, y3, 'x')
hold on
plot(x3, y3ode, '-')
hold off
axis([0 x3(end) 0 y3(1,1)])
ODE files for kinetic models

function ydot= odefileEK1 (T,y,p)
K=y(1);
%enter upper bounds for Vmf Vmr kmf kmr
K=(p(4)*p(1))/(p(3)*p(2)) %equilibrium constant
%initial aldose concentration (M)

%total enzyme g/L
A0=;
Et=;
Ae=A0/(1+K);
Ke=A0-Ae

km=((p(3)*p(4))/(p(4)-p(3)))*(1+((1/p(3))+(K/p(4)))*Xe) %Overall Michaelis constant (mol/L)
Vm=(1+(1/K))*((p(4)*p(1))/(p(4)-p(3))) %Overall velocity
ydot = [(-Et)*((Vm*(K-Ke))/(km+(K-Ke))) ];
%END PROGRAM

function ydot= odefileEK2 (T,y,p)
K=y(1);
%enter upper bounds for Vmf Vmr kmf kmr
K=(p(4)*p(1))/(p(3)*p(2)) %equilibrium constant
%initial aldose concentration (M)

%total enzyme g/L
A0=;
Et=;
Ae=A0/(1+K);
Ke=A0-Ae

km=((p(3)*p(4))/(p(4)-p(3)))*(1+((1/p(3))+(K/p(4)))*Xe) %Overall Michaelis constant (mol/L)
Vm=(1+(1/K))*((p(4)*p(1))/(p(4)-p(3))) %Overall velocity
ydot = [(-Et)*((Vm*(K-Ke))/(km+(K-Ke))) ];
%END PROGRAM

function ydot= odefileEK3 (T,y,p)
K=y(1);
%enter upper bounds for Vmf Vmr kmf kmr
K=(p(4)*p(1))/(p(3)*p(2)) %equilibrium constant
%initial aldose concentration (M)
%total enzyme g/L
A0=;
Et=;
Ae=A0/(1+K);
Ke=A0-Ae

km=((p(3)*p(4))/(p(4)-p(3)))*(1+((1/p(3))+(K/p(4))) Xe) %Overall Michaelis constant (mol/L)
Vm=(1+(1/K))*((p(4)*p(1))/(p(4)-p(3))) %Overall velocity
ydot = [(-Et)*((Vm*(K-Ke))/(km+(K-Ke))) ];
%END PROGRAM

% Enzyme kinetics for XI at 60C (Xylose→Xylulose)

prompt1='What is the initial concentration of xylose in M?';
result1=input(prompt1);
prompt2='What is the total amount of enzyme (g/L)';
result2=input(prompt2);

X0=result1;
Et=result2;

Vmfx=2.31e-004; %velocity forward for xylose
kmfx=1.51; %Michaelis constant for forward reaction
kmry=1.25; %Michaelis constant for reverse reaction
Vmry= 5.26e-4; %Velocity reverse for xylose

Kx=(kmry*Vmfx)/(kmfx*Vmry) %equilibrium constant
Xe=X0/(1+Kx)
Ye=X0-Xe
kmx=((kmfx*kmry)/(kmry-kmfx))*(1+((1/kmfx)+(Kx/kmry))*Xe) %Overall Michaelis constant (mol/L)
Vmx=(1+(1/Kx))*((kmry*Vmfx)/(kmry-kmfx)) %Overall velocity (p=[Vmx;kmx;Xe;Et;Ye];

[T,y] = ode45(@ekineticsxylose,[0:1:300],[X0 0],[], p);
yx=y(:,1)-((.001*log(T)-0.0021)*X0*Et);

plot(T,yx, T,y(:,2))

function ydot=ekineticsxylose(T,y,p)
A=y(1); K=y(2);
%p=[Vmx;kmx;Xe;Et;Ye];
$$ydot = \begin{bmatrix} -((p(4))*((p(1)*(A-p(3)))/(p(2)+(A-p(3))))) \\ -((p(4))*((p(1)*(K-p(5)))/(p(2)+(K-p(5))))) \end{bmatrix};$$

% Enzyme kinetics for XI at 60C (Glucose→Fructose)

prompt1='What is the initial concentration of glucose in M?';
result1=input(prompt1);
G0=result1;
prompt2='What is the total amount of enzyme (g/L)';
result2=input(prompt2);
Et=result2;

Vmfg=7.1e-004; %velocity forward for glucose
kmfg=2.59; %Michaelis constant for forward reaction
kmrf=2.19; %Michaelis constant for reverse reaction
Vmrf=5.68e-4; %Velocity reverse for glucose

Kg=(kmrf*Vmfg)/(kmfg*Vmrf) %equilibrium constant
Ge=G0/(1+Kg)
Fe=G0-Ge;
kmg=((kmfg*kmrf)/(kmrf-kmfg))*(1+((1/kmfg)+(Kg/kmrf))*Ge) %Overall Michaelis constant (mol/L)
Vmg=(1+(1/Kg))*((kmrf*Vmfg)/(kmrf-kmfg)) %Overall velocity (mol/L)

p=[Vmg;kmg;Ge;Et;Fe];
[T,y] = ode45(@ekineticsglucose,[0:1:300],[G0 0],[], p);
yg=y(:,1)-((.0007*log(T) - 0.0012)*G0*Et);
plot(T,yg, T,y(:,2))

function ydot=ekineticsglucose(T,y,p)
A=y(1); K=y(2);
%p=[Vmg;kmg;Ae;Et;Fe];
ydot = [
(-((p(4))*((p(1)*(A-p(3)))/(p(2)+(A-p(3)))))
-((p(4))*((p(1)*(K-p(5)))/(p(2)+(K-p(5)))))
];
%END PROGRAM
Appendix B

Reactive-Extraction (RE) MatLab Code

%FIND REACTION PARAMETERS FOR SINGLE SUGAR at 60C
clc
prompt1='What is the pH of the aqueous solution at equilibrium?';
H=(10^-input(prompt1));
prompt2='What is the initial concentration of sugar in M?';
St=input(prompt2);
prompt3='What is the concentration of sugar in the organic phase in M?';
So=input(prompt3);
prompt4='What is the total concentration of N2B in M?';
BAt=input(prompt4);
prompt5='What is the total concentration of Chloride in M?';
Clt=input(prompt5);
prompt6='What is the aqueous concentration of Chloride in M?';
Claq=input(prompt6);
prompt7='What is the concentration of OH added in M?';
OH=input(prompt7);
Clo=Clt-Claq;
Sa=St-So;
K1=1.34e-11; %rate constant for N2B<-->N2B-+H+
K5=1005;
a=H/K1;
b=-(Claq/(Clo*K5));
\[
A = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1; 0 & 0 & 0 & 1 & 1 & 1; 0 & 0 & 1 & 1 & 0 & 1; 0 & 1 & 0 & 1 & 1 & 1; 1 & a & 0 & 0 & 0; 0 & 1 & 0 & b & 0 \end{bmatrix}; \\
B = [BA; Claq; So; OH; 0; 0]; \\
\]

\[
x = ((\text{inv}(A) * B)) \% [BA \ BA - BAS - BAS - BA\text{-Q} + BAS\text{-Q} +] \\
K2 = (x(4) * H) / (x(3)) \\
K3 = x(3) / (x(1) * Sa) \\
K4 = x(4) / (x(2) * Sa) \\
K6 = (x(6) * Claq) / (Clo * x(4)) \\
\% \text{END PROGRAM} \\
\]

% REACTIVE EXTRACTION MODEL FOR N2B and ALIQUAT 336 @ 60C

\[
\text{Clc} \\
\% \text{volumes used to convert all concentrations to mmol} \\
prompt1 = \text{"What is the volume of aqueous in L?";} \\
Va = \text{input}(\text{prompt1}); \\
prompt2 = \text{"What is the volume of organic in L?";} \\
Vo = \text{input}(\text{prompt2}); \\
\]

% composition of organic in mol
prompt3 = \text{"What is the total concentration of N2B in M?";} \\
BA = \text{input}(\text{prompt3}) * Vo; \\
prompt4 = \text{"What is the total concentration of Aliquat in M?";} \\
Clt = \text{input}(\text{prompt4}) * Vo; \\
\]

% composition of aqueous in mol
prompt5 = \text{"What is the total concentration of glucose in M?";} \\
Sgt = \text{input}(\text{prompt5}) * Va; \\
prompt6 = \text{"What is the total concentration of fructose in M?";} \\
Sft = \text{input}(\text{prompt6}) * Va; \\
prompt7 = \text{"What is the total concentration of xylose in M?";} \\
Sxt = \text{input}(\text{prompt7}) * Va; \\
prompt8 = \text{"What is the total concentration of xylulose in M?";} \\
Syt = \text{input}(\text{prompt8}) * Va; \\
prompt9 = \text{"What is the pH of the aqueous solution?";} \\
H = (10^{-((\text{input}(\text{prompt9})))}) * Va; \\
\]

% equilibrium constants converted from M to mol
K1 = 1.34e-11 * Va; \% (mol) \\
K2g = 4.47e-10 * Va; K2f = 2.73e-9 * Va; K2x = 6.07e-10 * Va; K2y = 1.24e-8 * Va; \% (mol) \\
K3g = 2.284e1 / Va; K3f = 4.12e1 / Va; K3x = 2.074e1 / Va; K3y = 4.692e1 / Va; \% (1/mol) \\
K5 = 1.005e3 * Va / Vo; \% (unitless)
\[ K_{6g} = 2.131e0 \times \frac{Va}{Vo}; \quad K_{6f} = 4.187e0 \times \frac{Va}{Vo}; \quad K_{6x} = 2.364e0 \times \frac{Va}{Vo}; \quad K_{6y} = 4.18e0 \times \frac{Va}{Vo}; \]

(unitless)

% start timer
tic
% set up syms to find mmol of components
syms x1 x2 x3 x4 x5 x6 x7 x8 x9 x10 x11 x12 x13 x14 x15 x16 x17 x18 x19 x20
% positive'
% 1BA 2BA, 3BA, 4BA, 5BAX, 6BAY, 7BAG-
% 8BAF, 9BAX, 10BAY-
% 11BA
% 12BAG-
% 13BAF
% 14BAX
% 15BAY-
% 16Ga, 17Fa, 18Xa, 19Ya, 20Cla
format short eng
% coefficients to simplify equations
A = K1/H;
B = H/K2g;
C = H/K2f;
D = H/K2x;
E = H/K2y;
% Solve system of equations for x's
S = solve(B*(x1 - x2) - x3 - x4 - x5 - x6 - x7 - x8 - x9 - x10 - x11 - x12 - x13 - x14 - x15 - x16, (A*(x1) - x2, (B*(x7) - x3, (C*(x8) - x4, (D*(x9) - x5, (E*(x10) - x6, ((x12*x20)/(K6g*(Clt - x20))) - x7, ((x13*x20)/(K6f*(Clt - x20))) - x8, ((x14*x20)/(K6x*(Clt - x20))) - x9, ((x15*x20)/(K6y*(Clt - x20))) - x10, (K5*x2*(Clt - x20)/x20) - x11, Sgt - x16 - x3 - x7 - x12, Sft - x17 - x4 - x8 - x13, Sxt - x18 - x5 - x9 - x14, Svt - x19 - x6 - x10 - x15, (K5*x2*(Clt - x20) - x16, (x4/(x1*K3g)) - x17, (x5/(x1*K3x)) - x18, (x6/(x1*K3y)) - x19, x11 + x12 + x13 + x14 + x15 - x20);
% convert x's to double form
r1 = double([S.x1])';
r2 = double([S.x2])';
r3 = double([S.x3])';
r4 = double([S.x4])';
r5 = double([S.x5])';
r6 = double([S.x6])';
r7 = double([S.x7])';
r8 = double([S.x8])';
r9 = double([S.x9])';
r10 = double([S.x10])';
r11 = double([S.x11])';
r12 = double([S.x12])';
r13 = double([S.x13])';
r14 = double([S.x14])';
r15 = double([S.x15])';
r16 = double([S.x16])';
r17 = double([S.x17])';
r18 = double([S.x18])';
r19 = double([S.x19])';
r20 = double([S.x20]);

anyzero = any([r1; r2; r3; r4; r5; r6; r7; r8; r9; r10; r11; r12; r13; r14; r15; r16; r17; r18; r19; r20] < 0);
% print solution to matrix that satisfies the solve matrix
r1(anyzero) = [];
r2(anyzero) = [];
r3(anyzero) = [];
r4(anyzero) = [];
r5(anyzero) = [];
r6(anyzero) = [];
r7(anyzero) = [];
r8(anyzero) = [];
r9(anyzero) = [];
r10(anyzero) = [];
r11(anyzero) = [];
r12(anyzero) = [];
r13(anyzero) = [];
r14(anyzero) = [];
r15(anyzero) = [];
r16(anyzero) = [];
r17(anyzero) = [];
r18(anyzero) = [];
r19(anyzero) = [];
r20(anyzero) = [];
A1 = [r1; r2; r3; r4; r5; r6; r7; r8; r9; r10; r11; r12; r13; r14; r15; r16; r17; r18; r19; r20; Clt - r20];

orgg = (A1(3) + A1(7) + A1(12)); % convert glucose organic to mol
aog = A1(16); % convert convert glucose aqueous to mol
orgf = (A1(4) + A1(8) + A1(13)); % convert fructose organic to mol
aqf=A1(17);%convert fructose aqueous to mol
orgx=(A1(5)+A1(9)+A1(14));%convert xylose organic to mol
aqx=A1(18);%convert xylose aqueous to mol
orgy=(A1(6)+A1(10)+A1(15));%convert xylulose organic to mol
aqy=A1(19);%convert xylulose aqueous to mol

glucoseorganic=orgg/Sgt*100
fructoseorganic=orgf/Sft*100
xyloseorganic=orgx/Sxt*100
xyluloseorganic=orgy/Syt*100

Cla=A1(20)/Clt*100
Clo=100-Cla
toc
%END PROGRAM
Appendix C

SIRE-BE MatLab Code

% Xylose SIRE with pre-isomerization
tic
format short eng

% prompts for aqueous composition
prompt1='What is the initial concentration of xylose in M?';
result1=input(prompt1);
prompt2='What is the aqueous volume in L?';
Va=input(prompt2);
X0i=result1*Va;
Y0=0;
prompt3='What is the total amount of enzyme (g/L)';
Et=input(prompt3);
prompt4='What is the pH of the aqueous solution?';
H=(10^-(input(prompt4)))*Va;

% Set organic parameters
prompt5='What is the volume of organic in L?';
Vo=input(prompt5);
prompt6='What is the total concentration of N2B in M?';
BAt=input(prompt6)*Vo;
prompt7='What is the total concentration of Aliquat in M?';
Clt=input(prompt7)*Vo;
prompt8='What is the total time of pre-isomerization in min?';
tisoend=input(prompt8);
prompt10='What is the total time of SIRE experiment in min?';
Tfinal=input(prompt10); % final time
prompt11='What is the sample rate for SIRE in min?';
step=input(prompt11);
icpreiso=[result1,Y0];
Vmfx=2.31e-004; %velocity forward for glucose
kmfx=1.51; %Michaelis constant for forward reaction
kmry=1.25; %Michaelis constant for reverse reaction
Vmry= 5.26e-4; %Velocity reverse for glucose

Kx=(kmry*Vmfx)/(kmfx*Vmry); %equilibrium constant
Xe=icpreiso(1,1)/(1+Kx);
Ye=icpreiso(1,1)-Xe;

kmx=((kmfx*kmry)/(kmry-kmfx))*((1/(kmry+Kx/kmry))*Xe); %Overall Michaelis constant (mol/L)
Vmx=(1+(1/Kx))*((kmry*Vmfx)/(kmry-kmfx)); %Overall velocity 

p=[Vmx;kmx;Xe;Et;Ye];
[t,x] = ode45(@ekineticsxylose,[0:1:tisoend],icpreiso,[],p);

xx=x(:,1)-((.001*log(T)-0.0021)*X0*Et);
Xisoend=xx(end)*Va; %moles glucose at the end of pre-isomerization

Yisoend=x(end,2)*Va; %moles of fructose at the end of pre-isomerization

orgx=0;
orgy=0;

Sxt=(Xisoend)+(orgx);
Syt=(Yisoend)+(orgy);

K1=1.34e-11*Va; % (mol)
K2g=4.47e-10*Va; K2f=2.385e-9*Va; K2x=6.07e-10*Va; K2y=1.24e-8*Va; % (mol)
K3g=2.284e1/Va; K3f=4.12e1/Va; K3x=2.074e1/Va; K3y=4.692e1/Va; % (1/mol)
K5=1.005e3*Va/Vo; %(unitless)
K6g=2.131e0*Va/Vo; K6f=4.187e0*Va/Vo; K6x=2.364e0*Va/Vo; K6y=4.18e0*Va/Vo; % (unitless)

A=K1/H; D=H/K2x; E=H/K2y; %simplify later expressions

syms x1 x2 x3 x4 x5 x6 x7 x8 x9 x10 x11 x12
% BA BA- BAG BAF BAG- BAF- BAQ BAGQ BAFQ Ga Fa Cla
%solve for x's
S=solve((BA-t-x2-x3-x4-x5-x6-x7-x8-x9-x10),(A*x1)-x2,(D*x5)-x6,((E*x6)-x4),(x8*(x12))/((K6x*(Cltx12)))-x5,((x9*x12)/(K6y*(Cltx12)))-x6,(K5*x2*(Cltx12))/x7,(Sx10-x3-x5)-x8,(Sy11-x4-x6)-x9,(x3/(x1*K3x))-x10,(x4/(x1*K3y))-x11,(Sx12-x7+8)-x12);
%convert x's to double form
r1=double([S.x1]);r2=double([S.x2]);

%find any r's that are less than zero and eliminate them from the matrix
anyzero = any([r1;r2;r3;r4;r5;r6;r7;r8;r9;r10;r11;r12] < 0);
\[ r_1(\text{anyzero}) = []; r_2(\text{anyzero}) = []; r_3(\text{anyzero}) = []; r_4(\text{anyzero}) = []; r_5(\text{anyzero}) = []; r_6(\text{anyzero}) = []; r_7(\text{anyzero}) = []; r_8(\text{anyzero}) = []; r_9(\text{anyzero}) = []; r_{10}(\text{anyzero}) = []; r_{11}(\text{anyzero}) = []; r_{12}(\text{anyzero}) = []; \]

% print solution to matrix that satisfies the solve matrix
\[
A_1 = [r_1; r_2; r_3; r_4; r_5; r_6; r_7; r_8; r_9; r_{10}; r_{11}; r_{12}; Clt - r_{12}];
\]

\[
\text{orgx} = (A_1(3) + A_1(5) + A_1(8)) / Vo; \quad \% \text{convert glucose organic to M for enzyme kinetics}
\]

\[
aqx = (A_1(4) + A_1(6) + A_1(9)) / Va; \quad \% \text{convert convert glucose aqueous to M for enzyme kinetics}
\]

\[
\text{orgy} = (A_1(4) + A_1(6) + A_1(9)) / Vo; \quad \% \text{convert fructose organic to M for enzyme kinetics}
\]

\[
aqy = (A_1(11)) / Va; \quad \% \text{convert fructose aqueous to M for enzyme kinetics}
\]

\[
\text{icsire} = [aqx, aqy]; \quad \% \text{set initial conditions for enzyme kinetic sugars}
\]

\[
sugar = \text{icsire};
\]

\[
\text{orgpreiso} = [\text{orgx}, \text{orgy}];
\]

% set ode solver parameters
\[
\text{steps} = \text{Tfinal} / \text{step}; \quad \% \text{number of steps for kinetic solver loop}
\]

\[
\text{int} = \text{Tfinal} / \text{steps}; \quad \% \text{set interval for time steps}
\]

\[
\text{tint} = 1; \quad \% \text{step between to and tf}
\]

\[
\text{ts} = 0; \quad \% \text{time start}
\]

\[
\text{org} = \text{zeros} \left( \text{steps}, 2 \right); \quad \% \text{set up matrix for organic values}
\]

\[
\text{torg} = \text{zeros} \left( \text{steps}, 1 \right); \quad \% \text{set up time matrix for organic values}
\]

\[
\text{aq} = \text{zeros} \left( \text{Tfinal}, 3 \right); \quad \% \text{set up matrix for aqueous values}
\]

\[
\text{sumsugar} = \text{zeros} \left( \text{steps}, 1 \right); \quad \% \text{total sugar matrix at each step in solver loop}
\]

\[
\text{species} = \text{zeros} \left( \text{steps}, 13 \right); \quad \% \text{matrix for boronic acid species at each step in solver loop}
\]

for \(q = 1: \text{steps}\)
\[
\text{to} = \text{int} \left( q - 1 \right); \\
\text{tf} = \text{int} \left( q \right); \\
\text{tspan} = [\text{to}: \text{tint}: \text{tf}]; \\
\text{Xe} = (aqx + aqy) / (1 + Kx); \\
\text{Ye} = (aqx + aqy) - \text{Xe}; \\
\text{p} = [\text{Vm}x; \text{km}x; \text{Xe}; \text{Et}; \text{Ye}]; \quad \% \text{parameters to pass to kinetic ode solver}
\]

\[
[T, y] = \text{ode45} \left( [@\text{ekineticsxylose}, \text{tspan}, \text{icsire}, [], \text{p}] \right); \quad \% \text{T= time, y= aqueous sugar in M}
\]

\[
aq = y(\text{end}, :); \quad \% \text{convert to mol for RE}
\]

\[
\text{Sxt} = aq(1) * \text{Va} + \text{orgx} * \text{Vo}; \quad \% \text{convert glucose total to mol for RE}
\]

\[
\text{Syt} = aq(2) * \text{Va} + \text{orgy} * \text{Vo}; \quad \% \text{convert fructose total to mol for RE}
\]

\[
\% \text{set solver for new sugar values at each step in solver}
\]

\[
\text{syms x1 x2 x3 x4 x5 x6 x7 x8 x9 x10 x11 x12}
\]

\[
\%
\%
\% \text{1BA 2BA 3BAX 4BAY 5BAX 6BAY 7BAQ 8BAFQ 9BAYQ 10Xa 11Ya 12Cla}
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anyzero = any([r1;r2;r3;r4;r5;r6;r7;r8;r9;r10;r11;r12] < 0);
r1(anyzero)==[];
r2(anyzero)==[];
r3(anyzero)==[];
r4(anyzero)==[];
r5(anyzero)==[];
r6(anyzero)==[];
r7(anyzero)==[];
r8(anyzero)==[];
r9(anyzero)==[];
r10(anyzero)==[];
r11(anyzero)==[];
r12(anyzero)==[];
AA=[r1;r2;r3;r4;r5;r6;r7;r8;r9;r10;r11;r12;Clt-r12];
orgx=(AA(3)+AA(5)+AA(8))/Vo;%convert glucose organic to M for enzyme kinetics
aqx=AA(10)/Va;%convert glucose aqueous to M for enzyme kinetics
ory=(AA(4)+AA(6)+AA(9))/Vo;%convert fructose organic to M for enzyme kinetics
aqy=AA(11)/Va;%convert fructose aqueous to M for enzyme kinetics
icsire=[aqx,aqy];%set initial conditions for enzyme kinetics
sugar=[sugar; y(2:size(y,1),:)];
ts=[ts; T(2:size(T,1))];
org(q,:)=orgx,orgy;
torg(q,:)=step*q;
sumsugar(q,:)=orgx*Vo+orgy*Vo+aqx*Va+aqy*Va;
species(q,:)=AA(:,1);
end
orgtime=[0;torg]+tisoend;
orgtotal=[orgpreiso;org]*Vo;
aqtotal=[sugar]*Va;
aqtime=ts+tisoend;
preisum=(xx+x(:,2))*Va;
totaltime=[t;torg+tisoend];
sumsugar=[preisum;sumsugar];
preiso=[xx*Va,x(:,2)*Va];
figure
plot(t,preiso,aqtime,aqtotal,orgtime,orgtotal,totaltime,sumsugar)
toc

BA=species(end,1)/BAt*100;
BA_neg=species(end,2)/BAt*100;
BA_X=species(end,3)/BAt*100;
BA_Y=species(end,4)/BAt*100;
BA_X_neg=species(end,5)/BAt*100;
BA_neg=species(end,6)/BAt*100;
BA_Q=species(end,7)/BAt*100;
BA_XQ=species(end,8)/BAt*100;
BA_YQ=species(end,9)/BAt*100;
Cl_a=species(end,12)/Clt*100;
Cl_o=species(end,13)/Clt*100;
BA_bound=BA+BA_Y+BA_X_neg+BA_Y_neg+BA_X+BA_Y;
Aqy=(aqy*Va)/X0i*100;
Aqx=(aqx*Va)/X0i*100;
Orgy=(orgy*Vo)/X0i*100;
Orgx=(orgx*Vo)/X0i*100;
%END PROGRAM

%Glucose SIRE with pre-isomerization

tic
format short eng

%prompts for aqueous composition
prompt1='What is the initial concentration of glucose in M?';
result1=input(prompt1);
prompt2='What is the aqueous volume in L?';
Va=input(prompt2);
G0i=result1*Va;
F0=0;
prompt3='What is the total amount of enzyme (g/L)';
Et=input(prompt3);
prompt4='What is the pH of the aqueous solution?';
H=(10^-(input(prompt4)))*Va;

%Set organic parameters

prompt5='What is the volume of organic in L?';
Vo=input(prompt5);
prompt6='What is the total concentration of N2B in M?';
BAt=input(prompt6)*Vo;
prompt7='What is the total concentration of Aliquat in M?';
Clt=input(prompt7)*Vo;
prompt8='What is the total time of pre-isomerization in min?';
tisoend=input(prompt8);
prompt10='What is the total time of SIRE experiment in min?';
Tfinal=input(prompt10);% final time
prompt11='What is the sample rate for SIRE in min?';
step=input(prompt11);
icpreiso=[result1,F0];

Vmfg=7.1e-004; %velocity forward for glucose
kmfg=2.59; %Michaelis constant for forward reaction
kmrf=2.19; %Michaelis constant for reverse reaction
Vmrf=5.68e-4; %Velocity reverse for glucose
\[ K_g = \frac{(km_f \cdot V_m f g)}{(km_f \cdot V_m rf)}; \text{%equilibrium constant} \]

\[ Ge = icprio(1,1)/(1+K_g); \text{%Equilibrium constant for glucose} \]

\[ Fe = icprio(1,1) - Ge; \text{%Equilibrium constant for fructose} \]

\[ kmg = \frac{(km_f \cdot km_r)}{(km_r - km_f)} \cdot (1 + \frac{1}{km_f} + \frac{K_g}{km_r}) \cdot Ge; \text{%Overall Michaelis constant (mol/L)} \]

\[ V_{mg} = \frac{1}{1 + \frac{1}{K_g}} \cdot \frac{(km_r \cdot V_m f g)}{(km_r - km_f)}; \text{%Overall velocity} \]

\[ p = [V_{mg}; kmg; Ge; Et; Fe]; \]

\[ [t,x] = \text{ode45(@ekineticsglucose}, [0:1:t_{isoend}], icprio, [], p); \]

\[ xx = x(:,1) - ((.0007*\log(t) - 0.0012) \cdot \text{result1} \cdot Et); \]

\[ G_{isoend} = xx(\text{end}) \cdot V_a; \text{%moles glucose at the end of pre-isomerization} \]

\[ F_{isoend} = xx(\text{end,2}) \cdot V_a; \text{%moles of fructose at the end of pre-isomerization} \]

\[ \text{orgg} = 0; \]

\[ \text{orgf} = 0; \]

\[ \text{Sgt} = (G_{isoend}) + \text{orgg}; \]

\[ \text{Sft} = (F_{isoend}) + \text{orgf}; \]

\[ K_1 = 1.34e-11 \cdot V_a; \text{%(mol)} \]

\[ K_2_g = 4.47e-10 \cdot V_a; K_2_f = 2.385e-9 \cdot V_a; K_2_x = 6.07e-10 \cdot V_a; K_2_y = 1.24e-8 \cdot V_a; \text{%(mol)} \]

\[ K_3_g = 2.284e1/V_a; K_3_f = 4.12e1/V_a; K_3_x = 2.074e1/V_a; K_3_y = 4.692e1/V_a; \text{%(1/mol)} \]

\[ K_5 = 1.005e3 \cdot V_a/V_o; \text{%(unitless)} \]

\[ K_6_g = 2.131e0 \cdot V_a/V_o; K_6_f = 4.187e0 \cdot V_a/V_o; K_6_x = 2.364e0 \cdot V_a/V_o; K_6_y = 4.18e0 \cdot V_a/V_o; \text{%(unitless)} \]

\[ A = K_1/H; B = H/K_2_g; C = H/K_2_f; \text{%simplify later expressions} \]

\[ \text{syms } x1 \ x2 \ x3 \ x4 \ x5 \ x6 \ x7 \ x8 \ x9 \ x10 \ x11 \ x12 \]

\[ \% BA BA- BAG BAF BAG- BAQ BAGQ BAFQ Ga Fa Cla \]

\[ \%solve for x's \]

\[ S = \text{solve(} (B*A-t2-x3-x4-x5-x6-x7-x8-x9-x1), (A*x1)-x2, (B*x5)-x3, ((C*x6) - x4), ((x8*(x12))/(K6_g*(Clt-x12)))-x5, ((x9*(x12))/(K6_f(Clt-x12)))-x6, (K5*x2*(Clt-x12))-x7, (Sgt-x10-x3-x5)-x8, (Sft-x11-x4-x6)-x9, (x3/(x1*K3_g))-x10, (x4/(x1*K3_f))-x11, (x7+x8+x9)-(x12)); \]

\[ \%convert x's to double form \]

\[ r1 = \text{double}([S.x1])'; r2 = \text{double}([S.x2]); \]

\[ r3 = \text{double}([S.x3]); r4 = \text{double}([S.x4]); r5 = \text{double}([S.x5]); r6 = \text{double}([S.x6]); r7 = \text{double}([S.x7]); r8 = \text{double}([S.x8]); r9 = \text{double}([S.x9]); r10 = \text{double}([S.x10]); r11 = \text{double}([S.x11]); r12 = \text{double}([S.x12]); \]

\[ \%find any r's that are less than zero and eliminate them from the matrix \]

\[ \text{anyzero} = \text{any}([r1; r2; r3; r4; r5; r6; r7; r8; r9; r10; r11; r12] < 0); \]

\[ r1(\text{anyzero}) = []; r2(\text{anyzero}) = []; \]

\[ r3(\text{anyzero}) = []; r4(\text{anyzero}) = []; r5(\text{anyzero}) = []; r6(\text{anyzero}) = []; r7(\text{anyzero}) = []; r8(\text{anyzero}) = []; \]

\[ r9(\text{anyzero}) = []; r10(\text{anyzero}) = []; r11(\text{anyzero}) = []; r12(\text{anyzero}) = []; \]

\[ %print solution to matrix that satisfies the solve matrix \]

\[ A1 = [r1; r2; r3; r4; r5; r6; r7; r8; r9; r10; r11; r12; Clt-r12]; \]
\[
\text{orgg} = \frac{(A1(3)+A1(5)+A1(8))}{Vo}; \quad \% \text{convert glucose organic to M for enzyme kinetics}
\]

\[
\text{aqg} = \frac{A1(10)}{Va}; \quad \% \text{convert convert glucose aqueous to M for enzyme kinetics}
\]

\[
\text{orgf} = \frac{(A1(4)+A1(6)+A1(9))}{Vo}; \quad \% \text{convert fructose organic to M for enzyme kinetics}
\]

\[
\text{aqf} = \frac{A1(11)}{Va}; \quad \% \text{convert fructose aqueous to M for enzyme kinetics}
\]

\[
\text{icsire} = [\text{aqg}, \text{aqf}]; \quad \% \text{set initial conditions for enzyme kinetic sugars}
\]

\[
\text{sugar} = \text{icsire};
\]

\[
\text{orgpreiso} = [\text{orgg}, \text{orgf}];
\]

\[
\% \text{set ode solver parameters}
\]

\[
\text{steps} = \frac{T\text{final}}{\text{step}}; \quad \% \text{number of steps for kinetic solver loop}
\]

\[
\text{int} = \frac{T\text{final}}{\text{steps}}; \quad \% \text{set interval for time steps}
\]

\[
\text{tint} = 1; \quad \% \text{step between to and tf}
\]

\[
\text{ts} = 0; \quad \% \text{time start}
\]

\[
\text{org} = \text{zeros} (\text{steps}, 2); \quad \% \text{set up matrix for organic values}
\]

\[
\text{torg} = \text{zeros} (\text{steps}, 1); \quad \% \text{set up time matrix for organic values}
\]

\[
\text{aq} = \text{zeros} (\text{Tfinal}, 3); \quad \% \text{set up matrix for aqueous values}
\]

\[
\text{sumsugar} = \text{zeros} (\text{steps}, 1); \quad \% \text{total sugar matrix at each step in solver loop}
\]

\[
\text{species} = \text{zeros} (\text{steps}, 13); \quad \% \text{matrix for boronic acid species at each step in solver loop}
\]

\[
\text{for} \ q = 1: \text{steps}
\]

\[
\text{to} = \text{int} \ast (q-1);
\]

\[
\text{tf} = \text{int} \ast q;
\]

\[
\text{tspan} = [\text{to} : \text{tint} : \text{tf}];
\]

\[
\text{Ge} = (\text{aqg} + \text{aqf})/(1 + \text{Kg});
\]

\[
\text{Fe} = (\text{aqg} + \text{aqf}) - \text{Ge};
\]

\[
\text{p} = \text{[Vm}g; \text{kmg}; \text{Ge}; \text{Et}; \text{Fe}]; \quad \% \text{parameters to pass to kinetic ode solver}
\]

\[
\text{[T, y]} = \text{ode}45 (@\text{ekineticsglucose}, (\text{tspan}), \text{icsire}, [], \text{p}); \quad \% \text{T=time, y=aqueous sugar in M}
\]

\[
\text{aq} = \text{y(}\text{end}, :); \quad \% \text{convert to mol for RE}
\]

\[
\text{Sgt} = \text{aq}(1) \ast \text{Va} + \text{orgg} \ast \text{Vo}; \quad \% \text{convert glucose total to mol for RE}
\]

\[
\text{Sft} = \text{aq}(2) \ast \text{Va} + \text{orgf} \ast \text{Vo}; \quad \% \text{convert fructose total to mol for RE}
\]

\[
\% \text{re-set solver for new sugar values at each step in solver}
\]

\[
\text{sym} \ \text{x1} \ \text{x2} \ \text{x3} \ \text{x4} \ \text{x5} \ \text{x6} \ \text{x7} \ \text{x8} \ \text{x9} \ \text{x10} \ \text{x11} \ \text{x12}
\]

\[
\% \ \text{BA-BA-BAF-BAF-BAQ-BAGQ-BAF-Ga Fa Cla}
\]

\[
\text{S = solve}((\text{BAt-x2-x3-x4-x5-x6-x7-x8-x9-x1}),(\text{A*x1})-\text{x2}, (\text{B*x5})-\text{x3}, ((\text{C*x6}-\text{x4}),(\text{x8}*(\text{x12}))/((\text{K6g}*(\text{(Cl-x12})))-\text{x5}),(\text{x9}*(\text{x12}))/((\text{K6f}*(\text{Cl-x12})))-\text{x6}, (\text{K5*x2}*(\text{Cl-x12}))-\text{x7}, (\text{Sgt-x10-x3-x5}-\text{x8}, (\text{Sft-x11-x4-x6})-\text{x9}),(\text{x3} *(\text{x1*K3g}))-\text{x10}),(\text{x4}*(\text{x1*K3f}))-\text{x11}, (\text{x7}+\text{x8}+\text{x9}))-\text{x12});
\]

\[
\text{r1} = \text{double}([\text{S.x1}])';
\]

\[
\text{r2} = \text{double}([\text{S.x2}])';
\]

\[
\text{r3} = \text{double}([\text{S.x3}])';
\]

\[
\text{r4} = \text{double}([\text{S.x4}])';
\]

\[
\text{r5} = \text{double}([\text{S.x5}])';
\]

\[
\text{r6} = \text{double}([\text{S.x6}])';
\]

\[
\text{r7} = \text{double}([\text{S.x7}])';
\]

\[
\text{r8} = \text{double}([\text{S.x8}])';
\]

\[
\text{r9} = \text{double}([\text{S.x9}])';
\]

\[
\text{r10} = \text{double}([\text{S.x10}])';
\]

\[
\text{r11} = \text{double}([\text{S.x11}])';
\]

\[
\text{r12} = \text{double}([\text{S.x12}])';
\]

\[
\text{anyzero} = \text{any}([\text{r1}; \text{r2}; \text{r3}; \text{r4}; \text{r5}; \text{r6}; \text{r7}; \text{r8}; \text{r9}; \text{r10}; \text{r11}; \text{r12}] < 0);
\]
r1(anyzero)=[ ]; r2(anyzero)=[ ]; r3(anyzero)=[ ]; r4(anyzero)=[ ]; r5(anyzero)=[ ]; r6(anyzero)=[ ]; r7(anyzero)=[ ]; r8(anyzero)=[ ]; r9(anyzero)=[ ]; r10(anyzero)=[ ]; r11(anyzero)=[ ]; r12(anyzero)=[ ];
AA=[r1; r2; r3; r4; r5; r6; r7; r8; r9; r10; r11; r12; Clt-r12];

orgg=(AA(3)+AA(5)+AA(8))/Vo;% convert glucose organic to M for enzyme kinetics
aqg=AA(10)/Va;% convert glucose aqueous to M for enzyme kinetics
orgf=(AA(4)+AA(6)+AA(9))/Vo;% convert fructose organic to M for enzyme kinetics
aqf=AA(11)/Va;% convert fructose aqueous to M for enzyme kinetics
icsire=[aqg, aqf];% re-set initial conditions for enzyme kinetics
sugar=[sugar; y(2:size(y,1),:)];
ts=[ts; T(2:size(T,1))];
org(q,:)= [orgg, orgf];
torg(q,:)= step*q;
sumsugar(q,:)= (orgg*Vo+orgf*Vo+aqf*Va+aqg*Va);
species(q,:)= AA(:,1);
end
orgtime=[0; torg]+tisoend;
orgtotal=[orgpreiso; org]*Vo;
aqtotal=[sugar]*Va;
aqtime=ts+tisoend;
preisosum=(xx+x(:,2))*Va;
totaltime=[t; torg+tisoend];
sumsugar=[preisosum; sumsugar];
preiso=[xx*Va, x(:,2)*Va];

figure
plot(t, preiso, aqtime, aqtotal, orgtime, orgtotal, totaltime, sumsugar)
toc

BA=species(end,1)/BAt*100;
BAneg=species(end,2)/BAt*100;
BAG=species(end,3)/BAt*100;
BAF=species(end,4)/BAt*100;
BAGneg=species(end,5)/BAt*100;
BAFneg=species(end,6)/BAt*100;
BAQ=species(end,7)/BAt*100;
BAGQ=species(end,8)/BAt*100;
BAFQ=species(end,9)/BAt*100;
Cla=species(end,12)/Clt*100;
Clo=species(end,13)/Clt*100;
BAbound=BAG+BAF+BAGneg+BAFneg+BAGQ+BAFQ;
Aqf=(aqf*Va)/G0i*100;
Aqg=(aqg*Va)/G0i*100;
Orgf=(orgf*Vo)/G0i*100;
Orgg=(orgg*Vo)/G0i*100;

%END PROGRAM
Appendix D

Xylulose Dehydration to Furfural MatLab Code

function [estimates, ssetotal] = furfuralfitsimplifiedmodel(x,y);

%x=time in hrs. x1-x4 are for time points for 4 sets of experimental data
%y=concentrations in M. y1-y4 are for concentrations for 4 sets of experimental data
x1 = [ ];
% [A] (mmole/liter) [B] (mmole/liter) [C] (mmole/liter)
y1 = [ ];

x2=[ ];
y2=[ ];
x3=[ ];
%y3=[];
x4=[ ];
y4=[ ];

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tic
model = @ode;
options=gaoptimset('Generations', 3000, 'TolFun', 1e-9, 'TolCon', 1e-8);
%odds are activation energy and evens are A, Ea is J/mmol(kJ/mol) and A is in 1/hr
%for first order and 1/mMhr for second order
lb=[];
ub=[];
[estimates, ssetotal, exitflag, output] = ga(model, 6, [], [], [], [], lb, ub, [], options)

function [ssetotal] = ode(params)
% odds are activation energy and evens are A
p(1) = params(1);       p(2) = params(2);

function [t1ode, y1ode] = ode15s(@(t,y)odefilek6sansxylose1(t,y,p), x1, y1(1,:));
function [t2ode, y2ode] = ode15s(@(t,y)odefilek6sansxylose2(t,y,p), x2, y2(1,:));
function [t3ode, y3ode] = ode15s(@(t,y)odefilek6sansxylose3(t,y,p), x3, y3(1,:));
function [t4ode, y4ode] = ode15s(@(t,y)odefilek6sansxylose4(t,y,p), x4, y4(1,:));

FittedCurve1 = y1ode;
ErrorVector1 = (y1 - FittedCurve1);
sse1 = sum(sum(ErrorVector1.^2));

FittedCurve2 = y2ode;
ErrorVector2 = (y2 - FittedCurve2);
sse2 = sum(sum(ErrorVector2.^2));

FittedCurve3 = y3ode;
ErrorVector3 = (y3 - FittedCurve3);
sse3 = sum(sum(ErrorVector3.^2));

FittedCurve4 = y4ode;
ErrorVector4 = (y4 - FittedCurve4);
sse4 = sum(sum(ErrorVector4.^2));

ssetotal = sse1 + sse2 + sse3 + sse4;
end
figure(1)
plot(x1, y1, 'x')
hold on
plot(x1, y1ode, '-')
hold off
axis([0 x1(end) 0 215])

figure(2)
plot(x2, y2, 'x')
hold on
plot(x2,y2ode,'-')
hold off
axis([0 x2(end) 0 215])

figure(3)
plot(x3,y3,'x')
hold on
plot(x3,y3ode,'-')
hold off
axis([0 x3(end) 0 215])

figure(4)
plot(x4,y4,'x')
hold on
plot(x4,y4ode,'-')
hold off
axis([0 x4(end) 0 215])

temp1=[t1ode,y1ode]
temp2=[t2ode,y2ode]
temp3=[t3ode,y3ode]
temp4=[t4ode,y4ode]
Ea=[estimates(1,1); estimates(1,3); estimates(1,5); estimates(1,7); estimates(1,9); estimates(1,11)]
A=[estimates(1,2); estimates(1,4); estimates(1,6); estimates(1,8); estimates(1,10); estimates(1,12)]
toc
end
%END PROGRAM