Abstract: A solid vegetable waste stream was subjected to dilute acid (HCl) pretreatment with the goal of converting the waste into a form that is amenable to biochemical processes which could include microbial lipids, biohydrogen, and volatile organic acids production. Specifically, this study was conducted to identify the most suitable pretreatment condition that maximizes the yield or concentration of sugars while minimizing the production of compounds which are inhibitory to microbes (i.e., furfural, hydroxymethylfurfural, and organic acids). Temperatures from 50–150 °C and HCl loading from 0–7 wt % were studied using an orthogonal central composite response surface design with eight center points. The effects of the variables under study on the resulting concentrations of sugars, organic acids, and furans were determined using the quadratic response surface model. Results indicated that the biomass used in this study contains about 5.7 wt % cellulose and 83.8 wt % hemicellulose/pectin. Within the experimental design, the most suitable pretreatment condition was identified to be at 50 °C and 3.5 wt % HCl. A kinetic study at this condition indicated process completion at 30 mins. that produced a hydrolyzate that contains 31.30 ± 0.44 g/L sugars and 7.40 ± 0.62 g/L organic acids. At this condition, a yield of ~0.47 g sugar/g of dry solid vegetable waste was obtained. The absence of furans suggests the suitability of the resulting hydrolyzate as feedstock for biochemical processes. The results suggested that the sugar concentration of the pretreated biomass is highly affected by the presence of other compounds such as amines, amino acids, and proteins. The effect however, is minimal at low levels of HCl where the highest total sugar production was observed.

Keywords: onion wastes; lignocellulosic biomass; organic acids; microbial inhibitors; furans

1. Introduction

In the United States, there are about 1900 facilities that comprise the fruit and vegetable processing industry with a combined annual revenue of about $74 billion [1]. These entities generate large volumes of wastewater and solid wastes. During processing, more than 10% of the fruit or vegetable weight are discarded as solid wastes, which include peels, stalks, shells, leaves, pomace, rinds, pulp, cores, pits, stems, seeds, cuttings, and spoiled materials. While the discharge of the wastewater to municipal sewage system is possible for some operations, the disposal of solid wastes poses a more serious problem due to its highly perishable nature [2].
Some of the solid wastes generated during vegetable processing have high nutritional value and thus, may be used as animal feed. However, a large portion of this waste still needs to be treated or disposed in landfills. In addition, some vegetables such as onion processing wastes might not be suitable for utilization as animal feed [3]. As an alternative, these wastes could potentially be used as biomass feedstock for fuel and chemical production.

Studies indicated that onion wastes are suitable feedstock for production of biogas (a mixture of CH$_4$ and CO$_2$) through anaerobic digestion. Prior to digestion, the wastes are typically subjected to pretreatment to hydrolyze their cellulose and hemicellulose content and thus, liberating monomeric sugars. For example, Ligisan and Tuates Jr. [4] studied the optimization of the lime pretreatment of onion leaves and unmarketable onion bulbs for their potential use as feedstock for biogas production. They found that pretreated waste onion bulbs were highly biodegradable with high methane production potential. Technically however, the pretreated material can be used as feedstock for other biochemical processes than just biogas production (see Figure 1). These processes, which include aerobic fermentation to produce microbial lipids and anaerobic fermentation for production of alcohols; biohydrogen and volatile organic acids such as acetic acid, propionic acid, butyric acid; and others, might be the suitable options for waste valorization. In fact, due to today’s low prices of natural gas [5], biogas (a mixture of CH$_4$ and CO$_2$) production is not as economically attractive as before.

![Figure 1. Generalized processes for biochemical conversion of lignocellulosic biomass to fuels and chemicals.](image)

Biomass pretreatment are usually accomplished using acid or alkali, with acid pretreatment (i.e., using HCl and H$_2$SO$_4$) often deemed as one of the more promising method for industrial implementation [6]. During acid pretreatment, however, products that are known to inhibit microbial growth and metabolic activities are formed in addition to sugars. These inhibitory compounds, which include furans [i.e., hydroxymethylfurfural (HMF) from the degradation of hexoses and furfural from the degradation of pentoses], carboxylic acids (from depolymerization of hemicellulose and further degradation of furans), and phenolic compounds (e.g., vanillin from degradation of lignin), could be problematic during the fermentative conversion step of the pretreated feedstock. Thus, acid pretreatment conditions are usually chosen or optimized with the concentration of the above-mentioned inhibitory compounds as one of the major factors. The overall schematic for acid pretreatment of lignocellulosic biomass is presented in Figure 2 [6].

![Figure 2. Products from acid pretreatment of lignocellulosic biomass.](image)
In this study, a solid waste stream from an industrial vegetable processor was subjected to acid (HCl) pretreatment at different acid loadings (0–7 wt %), and temperatures (50–150 °C) with the aim of finding a condition that maximizes sugar yield and minimizes inhibitory compound formation. HCl was chosen as the acid pretreatment agent to eliminate the introduction of sulfur in the pretreated waste (if H₂SO₄ was used), which could affect the microbial consortium (i.e., proliferation of sulfur-degrading microbes [7]) during the downstream fermentative process.

2. Materials and Methods

2.1. Chemicals

All chemicals (sugar standards, trifluoroacetic acid, KOH, HCl, and H₂SO₄) used in the experiments were purchased from Fisher Scientific (Pittsburgh, PA, USA), while the gases used for analyses were obtained from Red Ball Oxygen (Shreveport, LA, USA).

2.2. Waste Sample Preparation

The solid vegetable waste was obtained from Guidry’s Fresh Cuts in Arnaudville, LA. The waste consisted of approximately 80% green onion and a mix of celery, pepper, and carrots. The obtained samples were homogenized using a shearing blender and kept frozen at −20 °C until further use.

2.3. Waste Composition Determination

The solid vegetable waste obtained from the industrial processor was composed of solid and liquid fractions. The waste was found to contain an average of 4 wt % solids as determined by an Ohaus MB45 infrared heater (Ohaus, Pine Brook, NJ, USA) moisture determination balance. Analysis of a portion of the liquid fraction was conducted to determine the concentration of free sugars, organic acids and furans initially present as detailed in Section 2.6. Sample Analysis. The composition (cellulose and hemicellulose) of the solid vegetable waste was determined based on protocols developed elsewhere [8,9]. Samples were dried in an oven at 80 °C until constant weight followed by composition determination, which were conducted in triplicates.

The solid vegetable waste was also subjected to C, H, N, and protein analyses as follows: A portion of the solid vegetable wastes were dried using a Freezone 6 Plus freeze dryer (Labconco, Kansas City, MO, USA). The freeze-dried waste was then analyzed for its elemental composition particularly C, H, N, and S using a Vario Micro Cube (Elementar, Mt. Laurel, NJ, USA) equipped with temperature programmable desorption trapping column and thermal conductivity detector (TCD). The analysis was done at a temperature range of 1100–1250 °C with sample size ranging from 1 to 10 mg using helium as carrier gas. Dried vegetable wastes samples were also sent out for independent C, H, and N analysis (Midwest Microlab, Indianapolis, IN, USA) [10] and protein content determination (New Jersey Feed Lab Inc., Trenton, NJ, USA) [11].

The composition of the solid portion of the waste stream was determined by a combination of the methods established by Bauer and Ibáñez [8] and Gao et al. [9] for cellulose and hemicellulose, respectively. These methods were modified and simplified version of the NREL’s official protocol “Determination of structural carbohydrates and lignin in biomass” by Sluiter et al. [12]. The resulting sugars concentrations were converted to glucan, xylan, and arabinan content of the biomass using the equations below [9].

\[
\text{Glucan content (\%) = } \frac{\text{Mass of Glucose (g)}}{\text{Mass of Sample (g)}} \times 1.111 \times 100 \tag{1}
\]

\[
\text{Xylan or XGM content (\%) = } \frac{\text{Mass of Xylose or XGM (g)}}{\text{Mass of Sample (g)}} \times 1.136 \times 100 \tag{2}
\]
Xylan or XGM content (%) = \frac{\text{Mass of Xylose or XGM (g)}}{\text{Mass of Sample (g)}} \times 100 \quad (3)

In the above equations, 1.111 and 1.136 are factors to convert the masses of the sugar monomers to their corresponding polysaccharides. It should also be noted that for the analysis used in this study, galactose and mannose have the same response and retention times as xylose. Thus, the xylan fraction might be a combination of xylan, galactan, and mannan or XGM.

2.4. Pretreatment Optimization

2.4.1. Design of Experiment

Temperatures from 50 to 150 °C and HCl loading from 0 to 7 wt % were studied for the acid pretreatment of the vegetable processing wastes. These ranges of acid loading and temperature were based on commonly used conditions for acid pretreatment of biomass [13]. An orthogonal central composite (inscribed) response surface design with 8 center points was used as experimental design, giving a total of 16 treatment combinations. Total sugars, total furans, total organic acids concentration were considered as experimental responses. Triplicate runs were conducted for all treatment combinations.

2.4.2. Experimental Procedure

Experimental runs were conducted using a fixed amount of solid wastes, while varying the acid concentration according to the design of experiment. Using an Instatherm® block system (AceGlass Inc., Vineland, NJ, USA), samples were heated to the target temperature (as dictated by experimental design) and kept at that temperature for 2 h with 300 rpm of agitation. After 2 h, samples were cooled, filtered through a 0.45 µm syringe filter, titrated to a pH range of 5–6 using a KOH solution and were analyzed as detailed in Section 2.6. Sample Analysis.

2.5. Kinetic Experiments

Kinetic experiment was conducted on the selected suitable treatment combination that was identified from the pretreatment optimization. Several samples (7 total) at the selected HCl loading were heated to the selected temperature. Each sample was assigned a pretreatment time (10, 20, 30, 40, 50, 85, and 120 min) by which, when the assigned time was reached, the sample was taken out of the Instatherm® block system and was allowed to cool down to room temperature. The cooled samples were immediately filtered using a 0.45-micron syringe filter, titrated to a pH range of 5–6 and were stored at 4 °C until further analysis.

2.6. Sample Analysis

The samples obtained from the composition determination, pretreatment optimization, and kinetic studies were analyzed for sugars (glucose, fructose, xylose and arabinose), organic acids (acetic, lactic, propionic, and butyric acids) and furans (furfural and HMF) simultaneously using a HP 1100 liquid chromatograph (LC) system (Agilent, Santa Clara, CA). The LC system was equipped with a diode array detector (DAD) and was coupled to a SofTA Model 300S evaporative light scattering detector (ELSD) (Teledyne Isco, Lincoln, NE, USA). The analysis was conducted in isocratic mode using an aqueous mobile phase that contains 0.1% (v/v) trifluoroacetic acid at a flow rate of 0.5 mL/min and injection volume of 20 µL. The column was a Rezex ROA-Organic acid having dimensions of 300 × 7.8 mm with a guard cartridge system (Phenomenex, Torrance, CA, USA) operating at 60 °C. The organic acids, furfural and HMF were quantified using the DAD at 210 nm while the sugars were detected using the ELSD with drift tube set at 65 °C, and spray chamber at 20 °C.
3. Results and Discussion

The waste biomass used in this study was from a processing plant that typically process 80% green onion and a mixture of celery, pepper, and carrots. Individually, these vegetables were found to have free sugar content (in wt % of solids): ~2.2 for onions, 5.4–6.2 for carrots, 0.9–1.2 for celery, and 1.7–2 for peppers [14]. Onions, which made up the majority of the biomass used in this study, generally contain (in wt %) 17.2 cellulose, 15.7 hemicellulose, and 2.4 lignin [15]. In addition, these vegetables are also known to contain significant amount of pectin [15–18]. The low lignin content of this biomass is advantageous as it indicates low energy and equipment cost for physical pretreatment [19].

Despite having 96 wt % water, the vegetable waste is categorized as ‘solid waste’ by the industrial processor and as such, is being sent to landfill. For this study, the waste stream was subjected to HCl pretreatment without modification other than blending for homogenization and size reduction. The water component of the waste was analyzed to determine the initial concentration of free sugars, organic acids, and furans. Results showed that glucose was the only detectable free sugar initially present in the solid waste at a concentration of 5.48 ± 0.01 g/L. Lactic and acetic acids were also present at concentrations of 1.62 ± 0.03 g/L and 0.67 ± 0.01 g/L, respectively. No furan inhibitors were detected in the waste stream.

3.1. Waste Composition

The carbohydrate composition of the solid waste is presented in Table 1, which shows a content of about 5.7 wt % cellulose and since both hemicellulose and pectin are heteropolysaccharides [20], 83.8 wt % of the waste can be considered hemicellulose/pectin fraction.

| Component                  | % Weight        |
|----------------------------|-----------------|
| Cellulose                  | 5.68 ± 0.23     |
| Hemicellulose/Pectin       |                 |
| Glucan                     | 13.23 ± 0.07    |
| Xylan/XGM                  | 39.67 ± 3.02    |
| Arabinan                   | 30.94 ± 1.44    |
| Protein                    | 11.36 ± 0.03    |
| C                          | 39.63 ± 0.42    |
| H                          | 6.24 ± 0.29     |
| N                          | 1.76 ± 0.05     |

1 Analysis was conducted by New Jersey Feed Lab Inc., Trenton, NJ; 2 Average of independent analyses conducted by Midwest Microlab, Indianapolis, IN and Energy Institute of Louisiana.

3.2. Pretreatment Optimization

The acid pretreatment numerical optimization was performed through a response surface methodology using a quadratic response surface model to capture curvatures inherent to an optimal state (minima or maxima) between experimental response, Y, and the factors being investigated. The model is given by

\[ Y = \beta_0 + \sum_{i=1}^{i} \beta_i x_i + \sum_{i=1}^{i} \beta_{ii} x_i^2 + \sum_{i<j}^{i<j} \beta_{ij} x_i x_j + \epsilon, \]  

(4)

where, \( i \) or \( j \) represents the factors under consideration, \( \beta_0 \) is the constant term, \( \beta_i \) is the linear coefficient of factor \( i \), \( \beta_{ii} \) is the quadratic coefficient of factor \( i \), \( \beta_{ij} \) is the interactive effect coefficient of factors \( i \) and \( j \), and \( \epsilon \) is the random error [21]. For a two-factor optimization, the quadratic response surface model expansion will yield

\[ Y = \beta_0 + \left( \beta_{11} x_1 + \beta_{22} x_2 \right) + \left( \beta_{12} x_1 x_2 \right) + \epsilon, \]  

(5)
For this study, \( Y = \) total sugar or total organic acid or total furan concentrations, \( x_1 = \) HCl concentration, and \( x_2 = \) temperature. It should be noted that the total furan concentration only include furfural and HMF. The full quadratic model contains six terms and the final models for the concentration of total sugars, total organic acids, and total furans were determined by including only those factors and factor interactions which are statistically significant at a significance level of 0.05 or those with \( p \)-values less than 0.05.

The collected data were coded using a coding scale of \(-1\) to \(+1\) to determine which of the factors (acid concentration and temperature) and factor interaction significantly affect the responses. Factor coding is a linear transformation of the factor space coordinates that removes the unit of measure. It is very useful in determining how the response changes relative to a representative center of design, which is the intercept \( (\beta_0) \) [21]. The uncoded and coded data were then fitted/regressed using the quadratic response surface model. The resulting uncoded and coded coefficients are presented in Table 2. The uncoded coefficients are useful for ‘plug and chug’ determination of responses given uncoded values of the factors being studied. The coded coefficients, on the other hand, are useful for determining how the responses are affected by the factors being studied.

As indicated in Figure 2, the generally accepted route for acid pretreatment of lignocellulosics involves an initial depolymerization that produces sugars, organic acids, and phenolics. The sugars then undergo further degradation to produce HMF and furfural [6,22]. This route indicates that organic acids could either be derived from direct biomass hydrolysis or from the degradation of monomeric sugars. The above route will be used to discuss the trends obtained for the pretreatment optimization.

### Table 2. Coded and uncoded coefficients for experimental responses.

| Coefficient | Total Sugar | Total Furans | Total Organic Acids |
|-------------|-------------|--------------|---------------------|
|             | Coded       | Uncoded      | Coded              | Uncoded          |
| \( \beta_0 \) | -0.274      | -9.378       | -0.252             | -9.601           | -0.875          | -20.255         |
| \( \beta_1 \) | -0.593      | 10.687       | -0.523             | 1.290            | -0.323          | -6.619          |
| \( \beta_2 \) | 0.138       | 0.528        | 0.444              | 0.179            | 0.223           | 0.815           |
| \( \beta_{11} \) | -0.221      | -0.880       | -0.180             | -0.059           | 0.322           | 1.914           |
| \( \beta_{22} \) | NS          | NS           | -0.421             | -0.001           | NS              | NS              |
| \( \beta_{12} \) | -0.449      | -0.107       | -0.677             | -0.013           | -0.405          | -0.144          |
| RMSE        | 6.065       | 9.1741       | 0.3635             | 0.81             | 0.94            | 0.70            |
| \( R^2 \) (adjusted) | 0.81       | 0.94         | 0.70               |                   |                 |                 |

1 NS-statistically not significant at \( \alpha = 0.05 \).

3.2.1. Total Sugar Concentration

For total sugar concentration, the resulting model has an adjusted \( R^2 \) value of 0.81, indicating the adequacy of the model used. The coded coefficients showed that temperature has a positive influence on the response while the rest of the significant factors and factor interaction affect the response negatively. The negative influence of the HCl concentration on the total sugar concentration could possibly be due to acid-catalyzed degradation of sugars to furans (i.e., HMF and furfural) which is known to be favored at low pH values [23]. The same is true for the negative influence of the quadratic element of acid concentration and the interaction between the two variables.

The influence of the factors and factor interaction were also evident in the contour plot of the total sugar concentration versus HCl concentration and temperature as presented in Figure 3a. It can be seen from the figure that the total sugars can be maximized by increasing the temperature at low HCl concentrations. However, the reverse is true at higher HCl concentrations. This indicates that the positive linear influence of temperature predominates at low HCl concentrations. As the concentration of HCl increases, the results indicated that the factor interaction have greater (negative) influence than the linear (positive) influence of temperature resulting to a net negative influence on the response.

At low HCl concentrations, the trend of increasing total sugar concentration as the temperature increases could be due to a faster rate of biomass hydrolysis than the sugar degradation at higher
temperatures. However, at higher HCl concentrations, increasing the temperature possibly resulted in increased reaction rates of both biomass hydrolysis and sugar degradation reactions (sugars to furans), with the latter likely being the dominant one. Thus, the trend was the opposite at high HCl concentrations.

![Figure 3. Contour plots of responses as a function of HCl concentration and temperature: (a) total sugar concentration, (b) total furan concentration, and (c) total organic acid concentration.](image)

3.2.2. Total Furan Concentration

The regression of total furan concentration indicated that all the terms of the quadratic response surface model are significant and resulted in an adjusted $R^2 = 0.94$. Table 2 presents the coded and uncoded coefficients of the model, which shows that only the linear element of temperature has a positive influence on the response (total furan concentration). This is surprising since it was anticipated that the trend of total furan concentration will be a complete opposite of that of the total sugar concentration. That is, the total sugar concentration is inversely proportional to the total furan concentration, which would make the effect of the factors and factor interaction (or the coded coefficients) a complete opposite of the results obtained. To further clarify this observation, we can compare the contour plots of total sugar (Figure 3a) and total furan (Figure 3b) concentrations. At low HCl concentrations and high temperatures, the feedstock was hydrolyzed to sugars to a certain extent, a fraction of which underwent degradation. A decrease in temperature at low HCl concentrations decreases the reaction rates and thus, resulted in lower sugar and furan concentrations. However, since the rates for both biomass hydrolysis and degradation reactions increases with acid concentration (thus the use of concentrated acids for pretreatment [24]), increasing the HCl concentration should have resulted in the same extent or higher sugar concentration. Given that the sugars will further degrade, and since sugar concentration decreased with HCl concentration, it was anticipated (due to Figure 2), that furans will increase with HCl concentration. Nevertheless, this was not the case and the results indicated an almost complete dependence of the total furan concentration to the total sugar concentration. One possible explanation is that the furans further degraded to organic acid since between the sugars → furans, and furans → organic acids, the former is the limiting reaction [25]. However, the results of the total organic acid concentration (Figure 3c) showed similar trend as the total furan concentration. This suggests that the trend of the sugar cannot be explained by the generally accepted route for acid pretreatment of biomass and requires other explanation.

Literature suggests that a component of the feedstock that could be tied into the results obtained are amines or amino acids. These are present in onions at significant quantities (5–7 wt %) [26], and analysis of the feedstock showed that it contains about 11 wt % proteins (see Table 1). In acidic environments, these amines could react with sugars through a process called the Maillard reaction, forming N-glycosides or N-substituted glycosylamines [27,28]. This reaction occurs at a more rapid rate at elevated temperatures [29]. During the analysis of the acid pretreated samples, no unknown major peaks were detected. However, the analytical method used was tailored for sugars, organic acids, and furans determination only. The product(s) of the Maillard reaction either did not make it through the process (filtered out) or were poorly chromatographed. The participation of this reaction during the acid pretreatment of solid vegetable waste would rationalize the sugar trend obtained. That is, the total sugar concentration increases with temperature at low HCl concentration, possibly because
there was not enough acid to catalyze the Maillard reaction. However, when the HCl concentration was increased, the effect of the Maillard reaction became significant. That effect was much more significant at the pretreatment condition with highest levels of both HCl concentration and temperature, and thus the behavior of the contour plot (Figure 3a) obtained for total sugar concentration. It should be noted however, that the participation of the Maillard reaction during the pretreatment process is only based on the composition of biomass used in this study in an effort to justify the results obtained.

3.2.3. Total Organic Acid Concentration

Onions are also known to contain organic acids such as oxalic, glutamic, tartaric, citric, and others [30,31], which have negative impact or inhibitory effect on microbes needed during biochemical processes. However, organic acids are not as toxic as furans obtained from biomass pretreatment, particularly furans and phenolic compounds [6]. Furthermore, as opposed to furans and phenolics, the inhibitory effect of organic acids can be easily mitigated by adjustment/control of process pH. Organic acids, which are also produced during anaerobic fermentation [32], have been utilized as substrates for fermentative microbial lipid production [33,34]. Thus, for this study, the organic acids can also be considered as another type of substrate (in addition to sugars) that can be used for fermentative production of fuels and chemicals.

Like the total sugar concentration, coded and uncoded model coefficients were estimated for total organic acid concentration and the resulting coefficients are presented in Table 2. The obtained coefficients correspond to an adjusted $R^2$ of 0.70. The coded coefficients indicated that HCl concentration and the interaction between HCl concentration and temperature have negative influence on the response (total organic acid concentration). On the other hand, temperature and the quadratic element of HCl concentration have a positive impact on the response. Except for the quadratic influence of HCl concentration, the general trend for total acid concentration within the experimental design is similar to the total sugar concentration. As previously mentioned, it was anticipated that the decrease in total sugar concentration, as HCl concentration was increased, will result in a higher total furan or total organic acid concentrations, or both. The results, however, suggested otherwise and it is speculated that the organic acids could have participated in the Maillard reaction as suggested by Kwon and Baek [35], or they could have reacted with the proteins (amines) through an amidation reaction [36,37]. Regardless of the fate of furans and organic acids, the results of this study suggest that the composition of biomass, particularly proteins, could tremendously affect the composition of the pretreated feedstock. Wood typically contains 0.03–0.59% N [38,39], a level which might not have an effect on the pretreatment route illustrated in Figure 2.

3.2.4. Principal Component Analysis

In addition to the contour plots, a principal component analysis (PCA) was performed using R statistical software (Figure 4). This analysis helped to visualize the relationship between total sugars, organic acids, and furans. They were also used to verify observations from the contour plots. PCA reduces data dimensionality via transformation into linearly uncorrelated vectors, in this case, from three-dimensional to two-dimensional.

The scree plot, which represent the fraction of total variance in the data [40], presented in Figure 4a indicates that a two-dimensional projection (as shown in Figure 4b) is sufficient to capture around 94.1% of the data. Figure 4b shows that total sugar and furan concentrations have very similar behavior and are highly correlated, as previously discussed. The total organic acid concentration, on the other hand, is also correlated to some degree. This is due to the quadratic element of acid concentration, which has an opposite influence on the total sugar and total organic acid concentrations discussed above.
were also present in the pretreated vegetable waste which could be problematic for the downstream processes. To this end, the data was analyzed using a minimax multi-objective optimization algorithm scripted in MATLAB® (9.1, The MathWorks Inc., Natick, MA, USA, 2016) as a first level optimization criterion to find the most suitable pretreatment condition. This minimax algorithm assigns a positive sign for the response(s) to be maximized and a negative sign to the response(s) to be minimized. The responses are then added, and the condition with the highest sum is chosen as the optimum condition.

### 3.2.5. Minimax Multi-Objective Function

The pretreated solid vegetable waste was intended to be used as feedstock for biochemical downstream processing to produce valuable products (i.e., microbial lipids, biohydrogen, and volatile organic acids). Thus, it is important to minimize the pretreatment products with unfavorable effects to microorganisms (i.e., organic acids and furans). The main aim of the optimization study was to identify an acid pretreatment condition that maximizes sugars while minimizing organic acids and furans. To this end, the data was analyzed using a minimax multi-objective optimization algorithm scripted in MATLAB® (9.1, The MathWorks Inc., Natick, MA, USA, 2016) as a first level optimization criterion to find the most suitable pretreatment condition. This minimax algorithm assigns a positive sign for the response(s) to be maximized and a negative sign to the response(s) to be minimized. The responses are then added, and the condition with the highest sum is chosen as the optimum condition.

### 3.2.6. Composition of Pretreated Feedstock

The result of the minimax optimization showed a suitable pretreatment condition at an acid concentration of 3.5 wt % and temperature of 150 °C. At this condition, the average concentrations of total sugar, organic acids, and furans were 35.30, 22.36, and 2.26 g/L, respectively. This is equivalent to a sugar yield of ~0.53 g per gram of vegetable waste (dry basis) and a total substrate (sugar + organic acids) yield of ~0.85 g per gram of waste. The composition of the pretreated solid vegetable waste is summarized in Table 3.

### Table 3. Composition of the pretreated solid vegetable wastes at 150 °C and 3.5 wt % HCl.

| Component          | Concentration (g/L) |
|--------------------|---------------------|
| Glucose            | 14.17 ± 0.23        |
| Xylose             | 12.53 ± 0.09        |
| Arabinose          | 8.60 ± 0.01         |
| Lactic acid        | 5.92 ± 0.53         |
| Acetic acid        | 6.05 ± 2.86         |
| Propionic acid     | 3.40 ± 2.40         |
| Butyric acid       | 6.99 ± 1.47         |
| Hydroxymethylfurfural | 0.30 ± 0.02     |
| Furfural           | 1.96 ± 0.16         |

### 3.2.7. Other Considerations

Significant quantities (~2.26 g/L) of furans (HMF and furfural) and organic acids (~22.36 g/L) were also present in the pretreated vegetable waste which could be problematic for the downstream fermentation conversion of the feedstock. Studies indicated that the presence of 2 g/L furfural or 1.5 g/L HMF completely inhibited the fermentative production of biohydrogen from pure sugars [42, 43]. It is also suggested that acetic acid can completely inhibit biohydrogen production at levels greater than...
10 g/L [42]. While 0.4 g/L of furfural was found to inhibit lipid production using Lipomyces starkeyi [44], organic acids were found to be suitable substrates for microbial lipid production [33,45]. As previously mentioned, however, the level of organic acids is not as critical as the level of furans in the pretreated waste. Thus, in the determination of the most suitable pretreatment condition, the concentration of furans was considered as the second level optimization criterion.

Furans can be removed or neutralized from the acid-pretreated feedstock prior to fermentation using different strategies including solvent extraction [46], adsorption and ion-exchange [47–49], overliming [50,51], and biological detoxification [52,53]. An alternative option is to utilize engineered microbes that can tolerate or even consume the furans [54,55]. However, a more desirable option is the reduction or elimination of these furan inhibitors during the pretreatment step. The results of the optimization study indicated that the pretreatment condition that uses 3.5 wt % HCl at 50 °C resulted in a total sugar and total organic acid concentrations of 31.3 ± 0.44 g/L and 7.40 ± 0.62 g/L, respectively, with no detectable furans. Among the pretreatment conditions tested, this condition had the second highest total sugar concentration obtained (the condition identified by minimax having the highest).

At this pretreatment condition, sugar yield was decreased by ~11% and the total concentration of organic acids was reduced to below 10 g/L. More importantly, however, is the reduction of furans below detectable levels. The formation of HMF and furfural are acid catalyzed reactions that also require heat. The results suggested that for the feedstock used in this study, 50 °C and 3.5 wt % HCl is a mild enough condition that prevented the formation of furans. According to Zhou et al. [56] temperatures from 119 °C to 130 °C are optimal for furans (particularly HMF) formation at different reaction solvents. A lower pretreatment temperature could also be a good way of reducing the operating cost (energy) of the process. Thus, the reduction of total organic acid to below 10 g/L and non-detectable level of furans makes the lower temperature condition very attractive and combined with a reduced energy requirement, provides a good tradeoff for the slight reduction in total sugar concentration. Hence, the lower temperature condition (3.5 wt % HCl concentration and 50 °C) was considered the most suitable pretreatment condition and was subjected to kinetic studies to determine pretreatment time.

3.3. Kinetic Experiments

The results of the kinetic experiments at 3.5 wt % HCl concentration and 50 °C are presented in Figure 5 which shows that the pretreatment was completed after 30 min. pretreatment. Beyond this time, the changes in the total concentrations were not statistically significant. It should be noted that, after 30 min., the resulting sugar and organic acid concentrations (28.78 ± 2.27 and 6.38 ± 0.56 g/L, respectively) are statistically similar to those obtained from pretreatment optimization at 3.5 wt % HCl concentration and 50 °C. These results suggest that the acid pretreatment of solid vegetable wastes can suitably be conducted using 3.5 wt % HCl loading at 50 °C for 30 min.

![Figure 5. Kinetics of acid pretreatment of solid vegetable wastes at 50 °C using 3.5 wt % HCl.](image-url)
4. Conclusions

A solid vegetable (mostly onions) waste stream was subjected to dilute HCl hydrolysis to determine the most suitable pretreatment condition. The biomass contains about 5.7 wt % cellulose and 83.8 wt % hemicellulose. This composition was different from literature values for green onions, which was likely due to the presence of other vegetable in the waste or to the differences in onion species used.

Temperature (50–150 °C) and HCl concentration (0–7 wt %) were the variables considered for optimization, which was aimed to maximize sugar concentration and minimize organic acid and furan concentrations. The total sugar, total organic acid, and total furan concentration responses were fitted to quadratic response surface model. Contour plots were produced using a combination of input parameters and responses determined by the central composite design of experiment. Additionally, a PCA was utilized to verify the plots and the trends obtained from the optimization study.

Results indicated that the composition of the feedstock could significantly affect the composition of the pretreated biomass and should be considered when identifying pretreatment parameters. The most suitable pretreatment condition was chosen based on the undetected levels of furans in the pretreated feedstock. Furans, particularly furfural and HMF, are known to inhibit microbial metabolism and thus could significantly affect the downstream biochemical process. On the other hand, organic acids as inhibitors can be easily mitigated by pH adjustment. They also have higher inhibitory threshold levels than furans. Based on these facts, the most suitable pretreatment condition was found to be at 50 °C and 3.5 wt % HCl. At this condition, the pretreatment has to be conducted for at least 30 min and would result in a pretreated feedstock that contains 31.3 ± 0.44 g/L sugars and 7.40 ± 0.62 g/L organic acids with no detectable furans.

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