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Kinetic Study on Heterotrophic Growth of *Acetobacterium woodii* on Lignocellulosic Substrates for Acetic Acid Production

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Abstract: Extensive research has been done on examining the autotrophic growth of *Acetobacterium woodii* with gaseous substrates (hydrogen and carbon dioxide) to produce acetic acid. However, only limited work has been performed on the heterotrophic growth of *A. woodii* using pure sugars or lignocellulosic feedstocks-derived sugars as substrates. In this study, we examine the growth kinetics and acetic acid production of *A. woodii* on glucose and xylose. While good growth was observed with glucose as substrate, no significant growth was obtained on xylose. Kinetic studies were performed in batch culture using different concentrations of glucose, ranging from 5 g/L to 40 g/L. The highest acetate production of 6.919 g/L with a product yield of 0.76 g acetic acid/g glucose was observed with 10 g/L glucose as initial substrate concentration. When testing *A. woodii* on corn stover hydrolysate (CSH) and wheat straw hydrolysate (WSH) formed after pretreatment and enzymatic hydrolysis, we found that *A. woodii* showed acetic acid production of 7.64 g/L and a product yield of 0.70 g acetic acid/g of glucose on WSH, while the acetic acid production was 7.83 g/L with a product yield of 0.65 g acetic acid/g of glucose on CSH. These results clearly demonstrate that *A. woodii* performed similarly on pure substrates and hydrolysates, and that the processes were not inhibited by the heterogenous components present in the lignocellulosic feedstock hydrolysates.

Keywords: heterotrophic growth; autotrophic growth; *Acetobacterium woodii*; glucose; xylose; lignocellulose feedstocks; acetic acid; wheat straw hydrolysate; corn stover hydrolysate

1. Introduction

Acetic acid is one of the most valuable, industrially important chemicals, with a global demand of 13 million tons of virgin acetic acid in the year 2015, which is forecasted to increase to approximately 18 million tons by 2020. The market price of acetic acid in North America in 2014 was around $2.3 billion, which is expected to rise up to 2.8 billion by 2019 [1,2]. This high demand is because of its myriad of applications as a raw material for many products, like paper coatings, latex emulsion resins used in the production of paints, adhesives, cellulose acetate fibers, textile finishing agents, cigarette filter tow, and plastics [3,4]. Currently acetic acid production is predominantly done using petrochemical feedstocks through various processes, of which methyl carbonylation process is the most commonly used process [5]. Other commonly applied approaches of acetic acid production include aerobic fermentation of substrates like ethanol using *Acetobacterium* species, where ethanol is converted to acetic acid with oxygen, mainly using two types of fermentation processes, a trickling process or a submerged fermentation [6]. However, these processes suffer from high production cost due to the

requirement of continuous purging of oxygen to maintain an aerobic environment for obligate aerobic microbes, besides the additional cost of ethanol or any other substrates used for the production of acetic acid, along with the cost for upgrading the acetic acid produced to a higher quality [5,6]. Due to the abovementioned drawbacks of the existing methods and rising environmental concerns, significant interest has been gained for producing acetic acid from renewable sources such as lignocellulosic sugars and syngas from gasification of renewable feedstocks—all by fermentation of these products into acetic acid by microbial bioprocesses [7]. The major group performing this reaction is a specialized group called acetogens [8–10].

Acetogens are a diverse group of strictly anaerobic microorganisms which are able to consume a wide variety of substrates, from simple and complex organic sugars—like hexoses, pentoses, acids, and alcohols—to gaseous substrates, like H_2, CO, and CO_2, to produce acetic acid as the major product [11–13]. Until date, there have been more than 100 acetogens described with different metabolic and phylogenetic traits [14–16]. The acetogens are divided into around 22 genera, of which the two genera, *Acetobacterium* and *Clostridium* are the most widely studied. Of the array of different acetogens, *Moorella thermoacetica*, *Clostridium ljungdahlii*, and *Acetobacterium woodii* are the three mainly studied acetogens, all of which have high product synthesizing capabilities. These acetogens mainly follow two patterns of growth, heterotrophic-utilizing organic substrates and autotrophic-utilizing gaseous substrates like H_2, CO_2, and CO, for energy and acetate synthesis [9,17–21]. However, less literature is available on the heterotrophic production of acetic acid by acetogens. Some of the reported studies include batch fermentation studies on *M. thermoacetica* [22] using different sugar substrates, such as glucose and xylose. These studies showed an initial consumption of xylose, followed by glucose, with a product yield of 0.76 g acetic acid/g xylose and 0.65 g acetic acid/g glucose. Similar research further done by Ehsanipour et al. [23] on the growth of *M. thermoacetica* on different hydrolyzed lignocellulosic substrates showed a product yield of around 0.71 g acetic acid/g sugarcane straw hydrolysate, and around 0.62 g acetic acid/g wheat straw hydrolysate, with no product inhibition by other components in the lignocellulosic feedstock. However, no literature was available on the heterotrophic growth of any of the other two acetogens, *C. ljungdahlii* and *A. woodii. A. woodii* has some obvious advantages for production of acetic acid as it is non-spore-forming, mesophilic, and more-or-less solely produces acetic acid during growth [10,24–26]. Detailed studies have been conducted on the autotrophic mode of growth of *A. woodii* using gaseous substrates like, H_2 and CO_2 [27–33]. However, the autotrophic growth of *A. woodii* suffers from some disadvantages, due to poor microbial biomass growth and low solubility of the gaseous substrates, especially hydrogen gas into the liquid phase, demanding specialized fermentation set-ups [34].

In this study, we explore the heterotrophic mode of growth by *A. woodii*, where ATP synthesis occurs by substrate level phosphorylation (SLP) of carbohydrates and other organic substrates, which are incompletely oxidized to produce acetate as the major end product. For *A. woodii*, highest ATP production of 4.3 ATP/mol glucose has been reported [35]. Of the 4.3 ATP molecules generated, 2 moles of ATP are synthesized by SLP during conversion of glucose to acetate, 2 moles of ATP by SLP during conversion of pyruvate to acetate, and 0.3 moles of ATP from a chemo-osmotic mechanism via the Na^+-dependent Rnf membrane complex [35]. Therefore, the heterotrophic conversion of substrates to acetate has been found to have an efficiency of 61%, making this process thermodynamically interesting and favorable for producing acetic acid [10,36].

Today, only very limited studies have been done on the heterotrophic growth of *A. woodii* for production of acetic acid using C6 and C5 sugars, such as glucose and xylose, which can be produced from lignocellulosic feedstocks. Lignocellulosic feedstocks, such as agricultural and forest residues, have been extensively studied for production of second-generation biofuels and biochemicals produced from the carbohydrate fraction of the lignocellulosic materials [37]. However, unlike food crops, lignocellulosic feedstocks contain lignin in addition to carbohydrates, which makes them more recalcitrant [38]. Therefore, a thermochemical treatment and enzymatic hydrolysis of the carbohydrate

polymer, to create monomeric subunits of glucose and xylose, needs to be applied upfront before the fermentation [39–43].

In this paper, we study growth and product kinetics of *A. woodii* on two pure sugar substrates, glucose and xylose, which are the two major sugars present in lignocellulosic feedstocks, and further apply these results to study the production of acetic acid by *A. woodii* on two different pretreated and enzymatically hydrolyzed lignocellulosic substrates, WSH and CSH. The ultimate aim of this study is to establish a bioprocess for production of acetic acid from sugars present in the lignocellulosic feedstocks.

2. Materials and Methods

2.1. Chemicals

All HPLC-grade reagents and calibration standards used in the experiments were obtained from Fisher Scientific (Pittsburg, PA, USA) and Sigma-Aldrich (St. Lois, MO, USA) respectively. D(+)-glucose (99+%) was procured from Fisher Scientific (Fair Lawn, NJ, USA) and D(+)-xylose (99+%) was procured from Acros Organics (Morris, NJ, USA). Yeast extract was purchased from US Biological (Swampscott, MA, USA). Aqueous sodium hydroxide (50 wt %) was purchased from Sigma-Aldrich. A gas cylinder containing a gaseous mixture of N_2/CO_2 (80:20) was purchased from Oxarc (Pasco, WA, USA) and was predominantly used for degassing purposes. Anhydrous sodium sulfide was obtained from Alfa Aesar (Ward Hill, MA, USA). All the vitamins required to make the vitamin mixture were ordered from Sigma-Aldrich, Alfa Aesar, Acros Organics, or Fisher Scientific.

2.2. Experimental Design

The experiments were designed to accomplish three main objectives: (i) to study the growth of *A. woodii* on two major sugars present in lignocellulosic feedstocks, glucose, and xylose as substrates; (ii) to study the effect of different substrate (glucose) concentrations (5–40g/L) on the growth kinetics and acetic acid production by *A. woodii*; and (iii) to compare with lignocellulosic feedstock substrates (WSH and CSH) for the growth of *A. woodii* and acetic acid production. The estimated and actual concentrations (measured using HPLC as discussed in Section 2.6.1) of sugars and acetic acid in the pure and lignocellulosic hydrolysates is shown in Table 1.

Table 1. Different concentrations of C6 (glucose), C5 (xylose) and lignocellulosic feedstock (WSH, CSH) sugars present in the batch experiments designed to test the growth of *A. woodii*. * All the experiments were performed in duplicates.

Feedstock	* Measured Concentration of Batch Feed before Inoculation (g/L)		
	Glucose	Xylose	Acetic Acid
5 g/L glucose	6.63	-	0
10 g/L glucose	9.87	-	0
20 g/L glucose	20.79	-	0
30 g/L glucose	31.23	-	0
40 g/L glucose	41.10	-	0
10 g/L xylose	-	10.28	-
WSH	18.58	13.45	3.31
CSH	16.66	10.02	5.16

2.3. Inoculum

A pure strain of *Acetobacterium woodii* DSM 1030 was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) (Braunschweig, Germany).

2.4. Substrate

Two different lignocellulosic feedstock hydrolysates, WSH and CSH, were used for examining the growth of *A. woodii*. Both feedstock materials were ground and pretreated at 25% dry matter using a wet explosion pretreatment method, as previously described by Biswas et al. [40]. In this treatment, the samples were exposed to a temperature of 190 °C for 30 min with an oxygen loading of 7.5% (*w*/*w*) of dry matter, in a pilot scale pressure vessel [40]. The pretreated slurry obtained after the pretreatment was diluted to 15% dry matter, and hydrolyzed using Novozymes Cellic CTec2 at 3.66 g enzyme per kg dry biomass. The process was performed at 50 °C and pH 5 for 4 days. The hydrolysates after enzymatic treatment were centrifuged at 4000 rpm for 60 min and filtered. These hydrolysates were stored at 4 °C until further use. The WSH contained 58.61 g/L glucose, 35.65 g/L xylose, 5.97 g/L cellulose, 2.55 g/L arabinose, 4.81 g/L acetic acid, and 0.86 g/L lactic acid. The CSH, on the other hand, contained 62.10 g/L glucose, 33.65 g/L xylose, 6.50 g/L cellulose, 5.20 g/L arabinose, 7.60 g/L acetic acid, and 1.03 g/L lactic acid. All batch experiments with pure sugars and lignocellulosic substrates were made in basic anaerobic (BA) medium [39] by adjusting the sugar concentrations to predetermined levels. Sodium sulfide (0.25 g/L) was used as reducing agent, while cysteine was omitted. The serum vials containing the BA medium were supplemented with 0.1% yeast extract and 1 vol % vitamin solution (DSMZ, Media 135, 141).

2.5. Batch Fermentation in Serum Vials

Batch fermentations with *A. woodii* were performed using 150 mL serum vials. BA medium (50 mL) was added into each of the serum vials, respectively. The vials were then purged with (80:20) N_2/CO_2 gas mixture for 15–20 min and sealed with rubber stopper (20 mm straight plug stopper, Wheaton, Millville, NJ, USA) and aluminum crimp caps. The vials containing the BA medium were sterilized by autoclaving at 121 °C for 20 min. Vitamin solution (DSMZ Media 141) and sodium sulfide (25 g/L stock solution) solution were each added at 1 vol % into the autoclaved serum vials after sterile filtering. To make different concentrations of glucose (5, 10, 20, 30, and 40 g/L) and xylose (10 g/L), respective stock solutions of sugar substrates were added into the autoclaved serum vials containing sterile medium, using sterile 0.45-micron filters. For the media preparation using lignocellulosic feedstocks, the total glucose concentration in the feedstock hydrolysates was diluted to ~15g/L using BA medium. Fifty milliliters of this mixture was added into the serum vials, purged with (80:20) N_2/CO_2 gas mixture for 15-20 minutes, and autoclaved. Vitamin solution and sodium sulfide solution were added in the same amount as mentioned above. The pH of the medium in the serum vials was adjusted to 7 $\pm$ 0.5 using sterile 5 N NaOH. After completing all the BA medium requirements, the serum vials were equilibrated to the incubation temperature of 30 $\pm$ 1 °C, before inoculation with 10% inoculum. The serum vials were incubated at 30 °C in a shaker at 150 rpm after culture inoculation. Samples (1 mL) were collected every 24 h using sterile syringes. The decrease in the pH due to acetic acid formation was adjusted daily to pH 7 $\pm$ 0.5 by addition of sterile 5 N NaOH. All procedures involving addition or removal from the serum vials were performed under sterile conditions on a clean bench. Samples collected from serum vials containing pure sugar substrates (glucose and xylose) were used to calculate OD and for HPLC analysis, while the samples collected from serum vials containing lignocellulosic feedstock hydrolysates were only sampled for HPLC analysis.

2.6. Analytical Methods

Samples collected from the serum vials at specific time intervals were used to measure pH for adjusting to optimal conditions, measure OD using a spectrophotometer, and to determine the concentrations of substrate and product formation using HPLC.

2.6.1. Measurement of Substrate Utilization and Product Formation Using HPLC Analysis

The samples collected from the batch serum vials were centrifuged at 10,000 rpm for 10 min, filtered using 0.2 µm filters, diluted, and analyzed using an UltiMate 3000 HPLC system (Dionex, Sunnyvale, CA, USA) equipped with Aminex 87H Column 250 × 4.6 mm (BioRad; Hercules, CA, USA) and a Shodex RI-101 refractive index detector. Dilute sulfuric acid (4 mM) in water was used as an eluent, flowing through the column with a constant flow rate of 0.6 mL/min. The column was maintained at a temperature of 60 °C during the analysis time of 30 mins/sample.

2.6.2. OD Measurement and Cell Dry Weight

Cell growth was monitored by measuring the optical density of the culture at 600 nm after specified time interval, using Jenway 6405 UV/Vis spectrophotometer (Jenway, Staffordshire, UK). The OD units were correlated to the dry weight of microbial biomass. Cell dry weight was obtained by serially diluting the microbial biomass, centrifugation at 8000 rpm for 20 min, and drying the centrifuge tubes at 60 °C overnight in a hot air oven. The difference in the centrifuge tubes containing the microbial biomass before and after drying was used to calculate the cell dry weight per unit wet weight. The correlation factor was then used to converted OD units at 600 nm to mg/L.

2.7. Calculations

Determination of Kinetic Constants

The maximum growth rate constant for five different concentrations of glucose were determined by integrating the following Equation (1):

$$\frac{dX}{dt} = e^{\mu t}\ [44] \quad (1)$$

In this equation, X_0 is the initial concentration of cells in mg/L, X is the concentration of cells at time t in mg/L, and μ_g is the growth rate constant in day^{-1}. This equation was used to plot a graph of $\ln(X/X_0)$ against time to determine the highest growth rate (μ_g/day) for every glucose concentration tested. The highest growth rate obtained for each of the different glucose concentrations were used to determine the overall kinetic constants, K_s (g/L), μ_{max} (day^{-1}), using Monod's growth kinetics (Equation (2)) [44]. This was done to estimate the optimal concentration of glucose ideal for achieving highest growth rate and acetic acid production by *A. woodii*, where μ_g is the specific growth rate of the microbe, μ_{max} is the maximum specific growth rate and K_s is the half-velocity constant.

$$\mu_g = \frac{(\mu_{max} S)}{(K_s + S)}\ [44] \quad (2)$$

The effect of substrate inhibition, if any, was studied using Equation (3), where the kinetic constants, K_s, K_i (inhibition constant) (mM), and μ_{max}, were estimated by plotting a graph.

$$\mu_g = \frac{\mu_{max} S}{\left(\left(K_s + S + \left(\frac{K_i^2}{S}\right)\right)\right.}\ [44] \quad (3)$$

Yield coefficients for gram of product formed per gram of substrate consumed for different concentrations of glucose and lignocellulosic feedstock sugars, WSH and CSH, were calculated using Equation (4), where P_0 and S_0 are the initial product and substrate concentrations in g/L, while P and S are the product and substrate concentrations at time t in g/L.

$$Y_{P/S} = \frac{(P - P_{0)}}{(S_0 - S)}\ [44] \quad (4)$$

3. Results

3.1. Growth of A. woodii on C6 (glucose) and C5 (xylose) Sugars

Growth studies of *A. woodii* on two different sugars, glucose and xylose, respectively, is shown in Figure 1. Microbial biomass growth of 82.95 mg/L was obtained when 10 g/L glucose was used as substrate, while growth on 10 g/L xylose was negligible. The initial increase in the cell growth and acetic acid, when using xylose as substrate, was due to the presence of traces of glucose in the inoculum added to the serum vials, as seen from the control without substrates. The microbe was further studied with different concentrations of glucose ranging from 5 to 40 g/L, to find the optimal glucose concentration for achieving maximum growth and product synthesis.

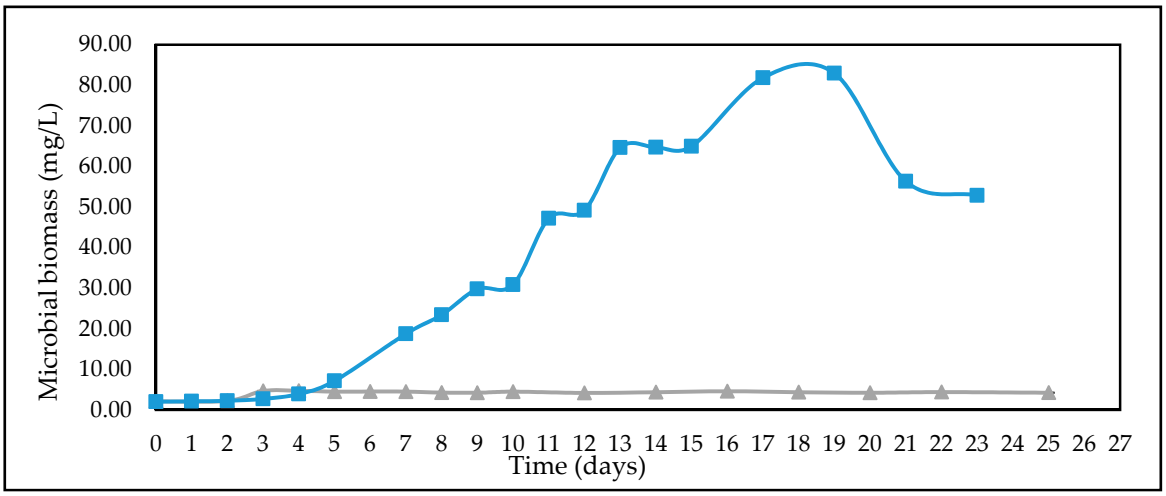

Figure 1. Growth of *A. woodii* (microbial biomass in mg/L) on 10 g/L glucose (■) and 10 g/L xylose (▲) (all standard deviations were less than 5% and cannot be seen in the graph).

3.2. Effect of Different Glucose Concentrations on the Kinetics of A. woodii

Figure 2 shows the growth of *A. woodii* on five different increasing concentrations of glucose, i.e., from 5 to 40 g/L glucose. As seen from Figure 2, the biomass concentration (mg/L) increases from 5 to 10 g/L glucose, followed by a gradual decrease in the cell concentration. A concentration of 10 g/L glucose was found to be the optimal concentration yielding highest microbial biomass, while higher concentrations of glucose were found to result in lower microbial growth. Simultaneous studies on the effect of different glucose concentrations on the production of acetic acid (Figure 3) by *A. woodii* showed similar results. It can be seen in Figure 3, there was an increase in acetic acid produced from 5 to 10 g/L glucose, with the highest acetic acid production of 6.92 g/L and microbial biomass of 82.95 mg/L for 10 g/L glucose, followed by a gradual decrease in the acetic acid produced with an increase in glucose concentrations from 20 to 40 g/L.

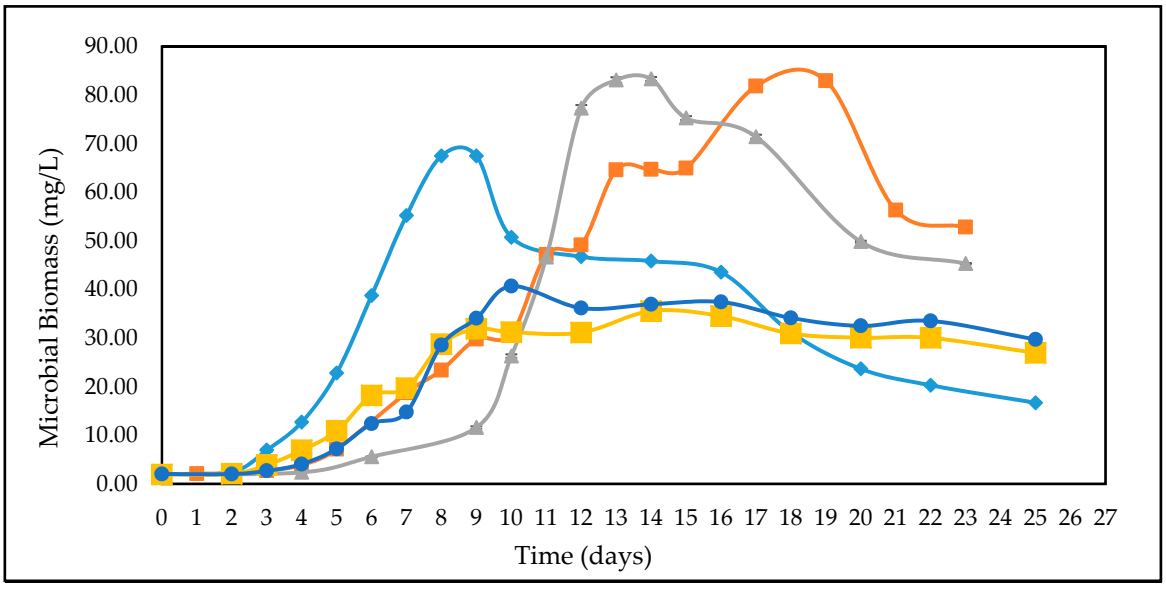

Figure 2. Effect of different concentrations of glucose on the growth of *A. woodii* over time measured in mg/L microbial biomass for: 5 g/L glucose (◆), 10 g/L glucose (■), 20 g/L glucose (▲), 30 g/L glucose (■), 40 g/L glucose (●). (all the standard deviations were less than 5% and cannot be seen in the graph).

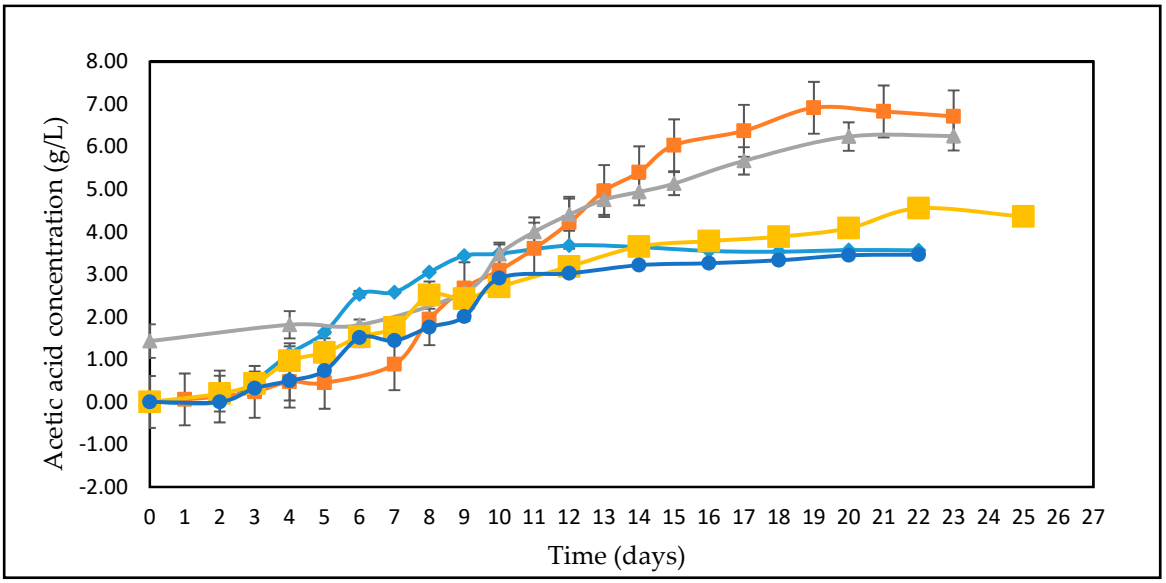

Figure 3. Effect of different concentrations of glucose on acetic acid production by *A. woodii* over time measured in g/L acetic acid produced for: 5 g/L glucose (◆), 10 g/L glucose (■), 20 g/L glucose (▲), 30 g/L glucose (■), 40 g/L glucose (●). (all the standard deviations were less than 10%).

3.3. Monod Growth Kinetics

Figure 4 represents the Monod kinetics for the growth of *A. woodii* on various concentrations of glucose as substrate. Maximum growth rates (μ_g) during exponential growth were calculated using Equation (1) and have been shown in Table 2 [32]. Equations (2) and (3) were further used to determine the kinetic constants where the maximum growth rates at different concentrations of glucose were plotted against respective glucose concentrations. The maximum growth rate (μ_{max}) was found to be 0.64 day^{-1} with a K_s value of 1.1 g/L concentration and an inhibition constant K_i of 123 mM (22.25 g/L). It can be observed, from Figure 4, that substrate inhibition occurs with an increase in the

concentration of glucose above 20 g/L (111 mM). This is further confirmed by an increase in the value of (S^2/K_i) from Equation (3), which increases with concentrations of glucose above 10g/L.

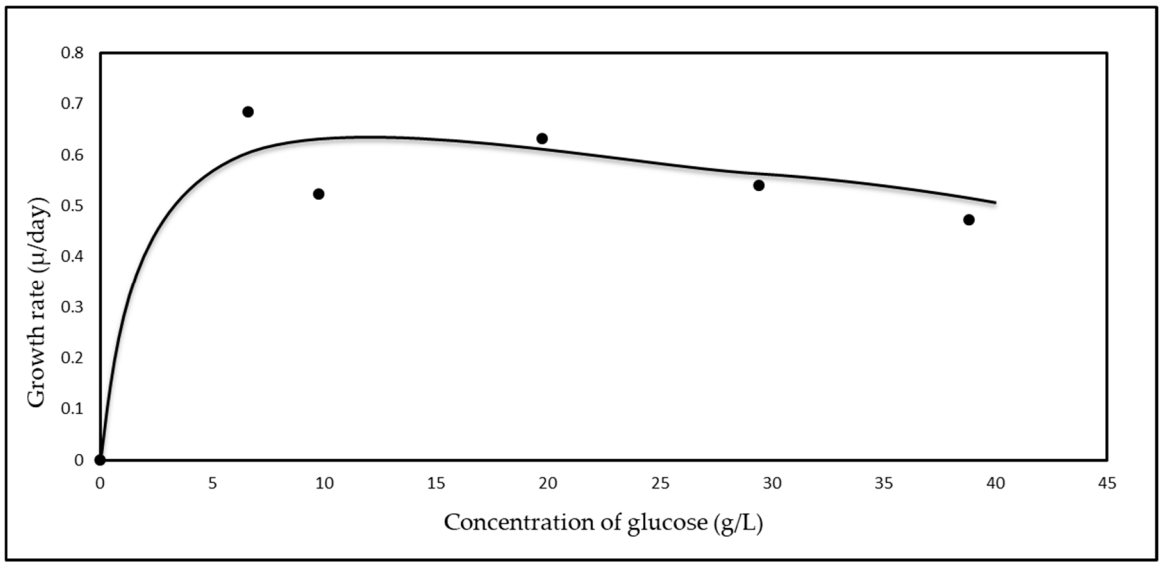

Figure 4. Growth rates of *A. woodii* at different concentrations of glucose for determination of the maximum growth rate and growth rate constants (K_s and K_i) using Monod kinetics.

Table 2. Maximum growth rate constants for different glucose concentrations calculated using Equation (1).

Glucose Concentration (g/L)	Maximum Growth Rate (μ_g) (day^{-1})
6.63	0.69
9.87	0.52
20.79	0.63
31.23	0.54
41.10	0.47

3.4. Studies on Lignocellulosic Feedstock Hydrolysates

The results from the batch experiments performed on different concentrations of glucose were used to determine the ideal glucose concentration for the lignocellulosic feedstock sugars. Both the hydrolysates were diluted to a total glucose concentration between 15 and 20 g/L. After a lag phase of 5 days, an increase in the glucose uptake and acetate production was observed for both the lignocellulosic sugar substrates, which is shown in Figures 5 and 6. Acetic acid production of 7.64 g/L was observed on WSH, and 7.83 g/L was found on CSH. No significant uptake of xylose present in WSH and CSH by *A. woodii* was observed.

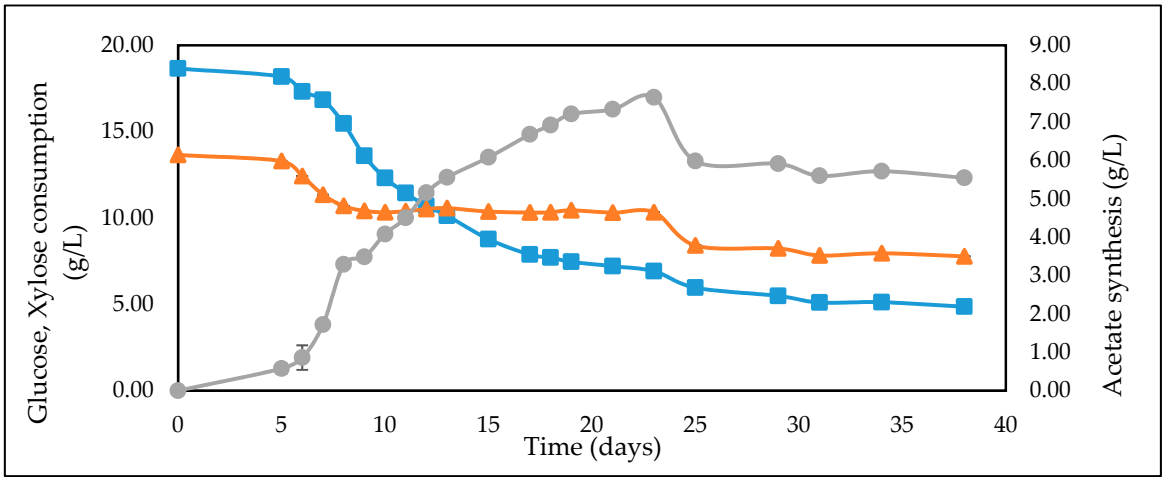

Figure 5. Time-based profile of: glucose uptake (g/L) (■), xylose uptake (g/L) (▲) and acetic acid production (g/L) (●) for WSH. (all the standard deviations were less than 5% and cannot be seen in the graph).

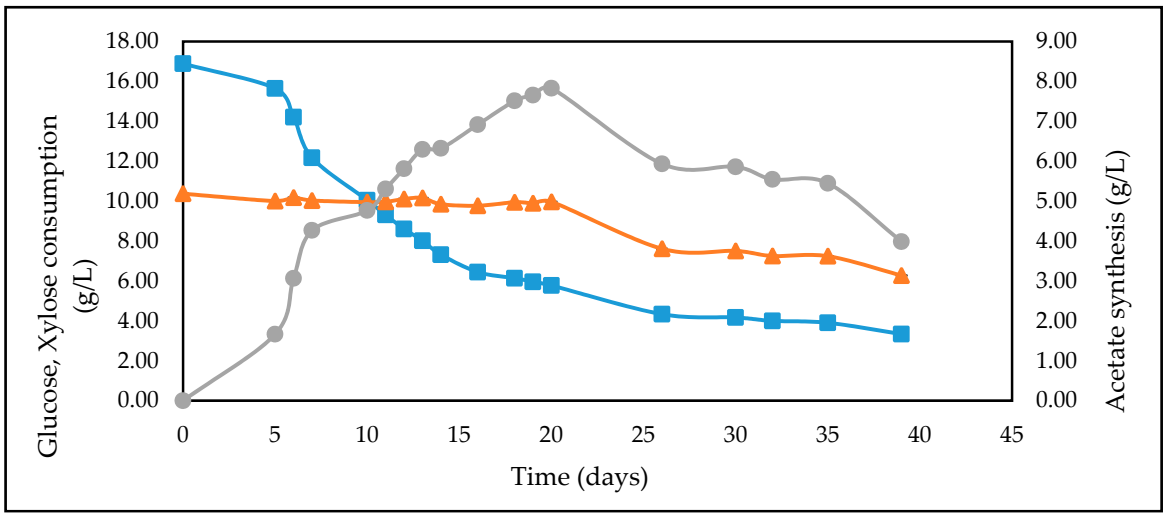

Figure 6. Time-based profile of: glucose uptake (g/L) (■), xylose uptake (g/L) (▲) and acetic acid production (g/L) (●) for CSH. (all the standard deviations were less than 5% and cannot be seen in the graph).

3.5. Calculation of Yield Coefficients

Yield coefficients were calculated using Equation (4). From Table 3, it can be seen that acetic acid production increases from 5 to 10 g/L glucose, after which there is a gradual decrease in the yield with an increase in the concentration of glucose from 20 to 40 g/L. The decrease in the acetic acid production for concentrations at and above 20 g/L might be because the higher concentration of substrate becomes inhibitory to the microbes. The results obtained from the experiments on pure glucose as substrate were used to determine the optimal concentration of glucose in the lignocellulosic substrates, and the amount of hydrolysates was adjusted to have a glucose concentration between 15 and 20 g/L in the final medium, i.e., ~18 g/L WSH and ~16 g/L CSH. A comparable product yield constant, $Y_{p/s}$, of 0.70 g acetate/g glucose was obtained for WSH and 0.65g acetate/g glucose for CSH, similar to pure substrate of 10 g/L glucose concentration.

Table 3. Calculation of yield coefficients for different glucose concentrations and lignocellulosic biomass sugars.

Concentration of Glucose (g/L)	$Y_{p/s}$
6.63	0.59
9.87	0.76
20.79	0.44
31.23	0.40
41.10	0.33
WSH (18.58)	0.70
CSH (16.66)	0.65

4. Discussions

Our studies discuss the potential of *A. woodii* for producing acetic acid from lignocellulosic feedstocks as substrates. To ensure that the experiments were performed using the right concentrations of substrates, the growth of *A. woodii* was first checked on pure sugars found in lignocellulosic biomass, i.e., glucose and xylose, to determine the growth kinetics and ideal concentration of sugars suitable for optimal product formation. We found that xylose was not an ideal substrate for growth and energy for *A. woodii*, in contrast to other studies done with homoacetogens such as *M. thermoacetica*. It was found that *A. woodii* showed the highest growth and acetate production at 10 g/L glucose, while glucose concentrations from 20 to 40 g/L were found to have lower yields, indicating an inhibitory effect on the microbe at high substrate concentrations, affecting the product yield. Therefore, when *A. woodii* was tested on actual lignocellulosic feedstock hydrolysates for its glucose conversion, the growth medium was adjusted to have a concentration between 15 and 20 g/L glucose. Interestingly, we found that growth under these conditions was similar to growth and acetic acid production on pure substrates. Overall, we can conclude that the maximum cell growth of *A. woodii* is found at a relatively low concentration of glucose (less than 20 g/L). One of the reasons for the substrate inhibition at higher sugar concentrations can be the fact that *A. woodii* in nature is mainly found in oligotrophic areas deficient in nutrients and, therefore, the strain might not be adapted to high concentrations of nutrients [38,39]. The higher acetic acid production in autotrophic mode, compared to heterotrophic mode, can be attributed to the production of 4.3 moles of ATP by *A. woodii* when growing heterotrophically, compared to 1.3 moles of ATP in the case of autotrophic growth. Therefore, deficiency of ATP molecules in autotrophic mode of growth might lead to higher rates of conversion when grown on gaseous substrates leading to higher production of acetic acid compared to the conditions during heterotrophic growth.

The results presented in this paper indicate a yield of 0.70 g acetate/g glucose from WSH and 0.65 g acetate/g glucose on CSH by *A. woodii*. This is comparable to the product yields reported by studies conducted using *M. thermoacetica* on lignocellulosic sugars under thermophilic conditions. Since *A. woodii* is a mesophilic, non-spore-forming homoacetogen, unlike *M. thermoacetica*, using this strain might be more cost effective and advantageous.

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Conflicts of Interest: The authors declare no conflict of interest.

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