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Optimization of pH as a strategy to improve enzymatic saccharification of wheat straw for enhancing bioethanol production

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Abstract

In this work, wheat straw (WS) was used as a lignocellulosic substrate to investigate the influence of pH on enzymatic saccharification. The optimum enzymatic hydrolysis occurred at pH range 5.8–6.0, instead of 4.8–5.0 as has been widely reported in research. Two enzymes cocktails, Celluclast® 1.5 L with Novozymes 188, Cellic® CTec2 and endo-1,4-β-xylanase, were used for the pH investigation over a pH range of 3.0–7.0. The highest concentration of total reduced sugar was found at pH 6.0 for all the different enzymes used in this study. The total reduced sugar produced from the enzymatic saccharification at pH 6.0 was found to be 7.0, 7.4, and 10.8 (g L^{-1}) for Celluclast® 1.5 L with Novozymes 188, endo-1,4-β-xylanase and Cellic® CTec2, respectively. By increasing the pH from 4.8 to 6.0, the total reduced sugar yield increased by 25% for Celluclast® 1.5 L with Novozymes 188 and endo-1 4-β-xylanase and 21% for Cellic® CTec2. The results from this study indicate that WS hydrolysis can be improved significantly by elevating the pH at which the reaction occurs to the range of 5.8 to 6.0.

Keywords: Wheat straw, Hydrolysis, pH effect, Sugar yield

Introduction

Environmental degradation and the universal need for energy have raised the demand for clean, easily available, and renewable energy sources to replace fossil fuel. The use of conventional fossil fuels as a major energy source has increased greenhouse gas emissions leading to global warming (Talebnia et al. 2010; Yang et al. 2013). Among the renewable energy sources, bioethanol has been of great interest in recent decades. There are many raw materials which can be used as resources for bio-ethanol production such as molasses, corn, and sugarcane. With the rising debate of food versus fuel, lignocellulosic waste presents a very good raw material for bioethanol production (Govumoni et al. 2013; Sarkar et al. 2012).

Bioethanol fuel production from lignocellulosic waste obtained from crops, wood, and agricultural residues represents a promising resource for a sustainable bioethanol fuel production due to the low cost and large quantity available worldwide (Avci et al. 2013; Talebnia et al. 2010). Among the variety of lignocellulosic materials, agricultural residues such as wheat straw (WS) stand as an important candidate for large scale bioethanol production. This can be attributed to its sustainability, abundance, and the large content of cellulose contrasted with a low lignin content (Qiu et al. 2017). According to statistics, WS which is a by-product from wheat production is one of the largest biomass feedstock in the world with a total production of approximately 690 kt in 2009, reaching 730 million tons in 2014 (Zheng et al. 2018). As a result, WS serves as a main appropriate lignocellulosic feedstock for bioenergy in the twenty-first century.

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WS cells mainly consist of three different polymers, namely, cellulose, hemicellulose, and lignin (de Assis Castro et al. 2017). The bioconversion of lignocellulosic to cellulosic biofuel via a reduced sugar (fermentable sugars) platform involves three key steps: the first step is pre-treatment, followed by enzymatic saccharification or catalytic conversion of reduced sugar and finally fermentation, the last step in the ethanol production line (Lan et al. 2013).

Enzymatic saccharification has been considered as a fundamental and the highest cost step in bioconversion of lignocelluloses. Few studies have been carried out using lignocellulosic substrates (instead of standard cellulose substrates) to find the optimum pH value for enzymatic hydrolysis (Lan et al. 2013). The majority of studies conducted on the enzymatic hydrolysis of lignocelluloses using *Trichoderma reesei* (i.e., Celluclast® 1.5 L) were performed at pH 4.8 and at a temperatures around 50 °C. These conditions were considered as the optimum condition for hydrolysis based on laboratory enzyme activities using model substrates, i.e., pure cellulose (Lan et al. 2013). The condition used for lignocelluloses enzymatic hydrolysis with endo-1,4-β-xylanase is quite similar to those commonly reported for Celluclast 1.5 L with Novozymes 188, which includes a temperature of 50 °C and pH 4.8–5.0 (Yang et al. 2015; Maitan-Alfenas et al. 2015).

Similarly, although the recommended pH range for Cellic Cellic® CTec2 by Sigma-Aldrich (Novozymes) is 5.0–5.5, pH 4.8 or 5.0 is the most commonly reported in the literature (Procentese et al. 2017; Sun et al. 2018). Celluclast® 1.5 L with Novozymes 188 and Cellic® CTec2 are among the most used enzymes for cellulose hydrolysis, whiles endo-1,4-β-xylanase is for hemicellulose hydrolysis (Avci et al. 2013; Oladi and Aita 2018; Jørgensen et al. 2007; Kumar et al. 2008).

Lignocellulosic substrates differ from pure cellulosic substrates in terms of physical and chemical compositions and structures. The presence of the hydrophobic lignin is considered a vital factor which inhibits the enzymatic hydrolysis of cellulose (Rajput and Visvanathan 2018). The mechanism by which lignin alters the hydrolysis process depends on the adsorption of cellulase on to lignin rather than cellulose via ionic bond interactions, hydrogen bond interactions, and hydrophobic interactions (Nakagame et al. 2011). To solve this problem, some researchers have modified the lignin surface using acid groups such as carboxylic and sulfonic to increase the hydrophilicity of the lignin (Nakagame et al. 2011). This reduces the non-productive (non-specific) binding to cellulase which limits the yield of cellulose hydrolysis during the biochemical reaction of the lignocellulosic biomass (Mansfield et al. 1999).

Lignin is considered as a phenolic polymer with three main hydroxycinnamoyl alcohols: sinapyl, coniferyl, and p-coumaryl alcohols. During the pre-treatment, these alcohols might be polymerized to guaiacyl, syringyl, and p-hydroxyphenyl moieties (Bonawitz and Chapple 2010). Both cellulases and hemicellulases are affected by lignin-derived phenols during enzymatic hydrolysis (dos Santos et al. 2018). Moreover, the exposed lignin present in the lignocellulosic biomass after pre-treatment affects the enzymes by absorbing them (Selig et al. 2007). Many binding mechanisms between enzymes and lignin have been suggested related to hydrophobic, electrostatic, and carbohydrate interactions (Sammond et al. 2014). pH is an important factor as it alters the surface hydrophobicity by inducing a surface charge, and this can also affect electrostatic interaction between lignin and cellulose (Lan et al. 2013).

The aim of the research in this paper was to investigate the optimal pH range for different commercial enzyme cocktails that gives maximal lignocellulosic saccharfication during the enzymatic hydrolysis for the WS as lignocelluloses substrates instead of the pure cellulosic substrate. The optimum pH for pure cellulosic substrate is established to be 4.8 which is also widely used as the optimum pH for lignocellulosic substrates during enzymatic hydrolysis. This study highlights that the optimum pH for pure cellulosic substrate (i.e., whatman filter paper) is not necessarily the optimum pH for lignocellulosic materials during enzymatic hydrolysis.

Experimental

Materials and methods

Celluclast® ® 1.5 L, Novozyme 188 (β-glucosidase) and endo-1,4-β-xylanase were purchased from Sigma-Aldrich Co., UK, while Cellic® CTec2 enzyme was garrulously provided by Novozymes Biotechnology Co., Ltd (Tianjin, China). The enzyme activities were measured according to the standard procedure (Ghose 1987). The Celluclast® ® 1.5 L and Cellic® CTec2 cellulase activities were and found to be 74 filter paper unit (FPU) mL^{-1} and 140 (FPU) mL^{-1}, respectively, the Novozyme activity was 760 cellobiase unit (CBU) mL^{-1}, and the endo-1,4-β-xylanas activity is 7700 Ug^{-1}. Sodium citrate buffer, sugar standards (glucose, xylose, galactose, mannose, arabinose, cellobiose), hydrochloric acid, sodium hydroxide, sodium azide, Whatman no. 1 filter paper strip, 3,5-dinitrosalicylic (DNS) acid, and Rochelle salt (sodium potassium tartrate tetrahydrate) were purchased from Fisher Scientific, UK. All experiments in this study were conducted using WS generously supplied from a local farm in Driffield, East Riding of Yorkshire, UK (Harvest Summer 2017).

Raw material preparation

To remove the surface dirt, the WS was washed with distilled water several times until the residue colour become white. The washed WS was then dissected into smaller parts using a knife blender (Luvele Power-Plus Blender | 2200w, UK) and milled using a laboratory ceramic desk grinder (Waldner, Biotech GMBH). The milled straw was then sieved (AS-200 control, Retsch GmbH) to get uniform particle sizes within a range more than 2000 to less than 250 μm and dried at 35 °C ± (2 °C) in a drying cabinet for 24 h. The moisture content was determined according to NREL protocol and found to be in the range of 8–10% (Sluiter et al. 2008).

Enzymatic hydrolysis assay

The dried WS biomass was enzymatically hydrolysed to release monomeric sugars from cellulosic materials. This was achieved using 1 g of dried WS in 50 mL of buffer solution (sodium citrate 0.05 M) allowing a total working volume of 50 mL. Prior to hydrolysis, 0.02% w/w sodium azide was added to the samples, before addition of the enzyme, to inhibit the microbial growth as this may consume the monomeric sugar produced and inhibit the enzyme's activity (da Costa Lopes et al. 2013; Qi et al. 2009). The samples were incubated for 60 min at (50 °C, 200 rpm) in a laboratory shaker/incubator (Orbital incubator SI 500, Stuart, UK). The hydrolysis proceeded under mild conditions (50 °C, 200 rpm) in the shaker/incubator for 94 h. The pH of the buffer solution was adjusted between 3.0 and 7.0 using 1 M sodium hydroxide and 1 M hydrochloric acid.

To investigate the pH effect on different enzymes, a cocktail of Celluclast® 1.5 L with an activity loading of 15 FPU g^{-1} DM and Novozyme 188 with an activity loading of 30 CBU g^{-1} DM was used. Additionally, xylanase enzyme with an activity loading of 1540 U g^{-1} DM was used for the pH investigation. The commercial cellulose enzyme cocktail Cellic® CTec2 with an activity loading of 15 FPU g^{-1} DM was also selected for these experiments. The enzymatic hydrolysis was carried for 94 h, but it was found that after 72 h, the total reduced sugar yield did not change; therefore, 72 h was used as the end of the hydrolysis instead of 94 h. Aliquots of the hydrolysate were withdrawn every 24 h from the hydrolysis to check the total reduced sugar residue. These aliquots were boiled for 5 minu to stop the enzymes activity and were then centrifuged (centrifuge 5702, Eppendorf, UK) at 4500 rpm for 5 min. The supernatants were sampled for total reduced sugar analysis using 3,5-dinitrosalicylic acid (DNS) reagent as described below (Miller 1959). All the hydrolysis experiments were carried out in triplicate to ensure reproducibility.

Analytical methods

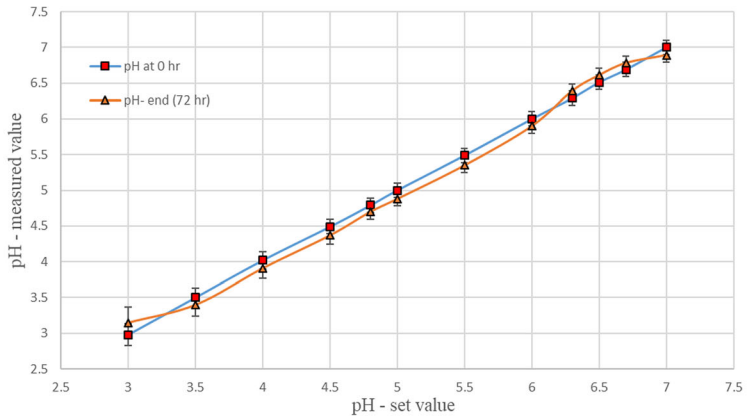
The raw WS carbohydrate composition, reduced sugar yield, and carbohydrate composition in different WS samples were determined with the help of standard laboratory analytical procedure. The details of the analysis are as follows:

Raw wheat straw composition using HPLC

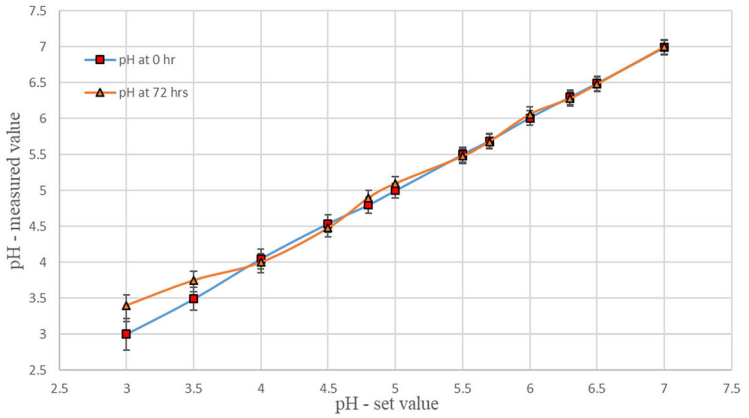
The carbohydrate composition of raw WS was determined by the NREL standard protocol (Sluiter et al. 2010). Oven-dried WS (0.3 g) was hydrolyzed with 3 mL of 72% sulfuric acid for 60 min at 30 °C in a water bath. The samples were then diluted with 84 mL of deionized

Table 1 Measured pH values before and after addition of the enzymes and adjusted

	pH after adding the enzymes			pH adjusted		
pH before adding the enzymes	Celluclast® 1.5 L + Novozymes 188	endo-1,4-β-xylanase	Cellic® CTec2	Celluclast® 1.5 L + Novozymes 188	endo-1,4-β-xylanase	Cellic® CTec2
3.00	3.55 ± 0.1	3.41 ± 0.07	3.61 ± 0.08	3.00 ± 0.01	2.99 ± 0.01	3.00 ± 0.01
3.50	3.96 ± 0.07	3.85 ± 0.05	4.0 ± 0.1	3.49 ± 0.01	3.5 ± 0.01	3.50 ± 0.01
4.00	4.40 ± 0.08	4.32 ± 0.08	4.42 ± 0.07	4.05 ± 0.01	4.02 ± 0.01	4.00 ± 0.01
4.50	4.79 ± 0.05	4.79 ± 0.06	4.70 ± 0.09	4.52 ± 0.01	4.49 ± 0.01	4.50 ± 0.01
4.80	5.10 ± 0.07	5.10 ± 0.06	5.20 ± 0.05	4.8 ± 0.01	4.79 ± 0.01	4.80 ± 0.01
5.00	5.24 ± 0.07	5.20 ± 0.06	5.18 ± 0.05	5.00 ± 0.01	5.00 ± 0.01	5.03 ± 0.01
5.50	5.70 ± 0.05	5.68 ± 0.04	5.63 ± 0.03	5.50 ± 0.01	5.49 ± 0.01	5.48 ± 0.01
5.70	5.88 ± 0.05	5.81 ± 0.02	5.80 ± 0.03	5.69 ± 0.01	5.70 ± 0.01	5.70 ± 0.01
6.00	6.15 ± 0.02	6.15 ± 0.02	6.13 ± 0.03	6.01 ± 0.01	6.00 ± 0.01	5.99 ± 0.01
6.30	6.43 ± 0.04	6.40 ± 0.02	6.39 ± 0.02	6.30 ± 0.01	6.29 ± 0.01	6.29 ± 0.01
6.50	6.60 ± 0.02	6.59 ± 0.01	6.55 ± 0.02	6.49 ± 0.01	6.51 ± 0.01	6.49 ± 0.01
6.70	7.10 ± 0.02	6.77 ± 0.01	6.75 ± 0.03	7.00 ± 0.01	6.69 ± 0.01	7.00 ± 0.01
7.00	7.20 ± 0.03	7.05 ± 0.01	7.03 ± 0.01	6.99 ± 0.01	7.00 ± 0.01	7.03 ± 0.01



(a)



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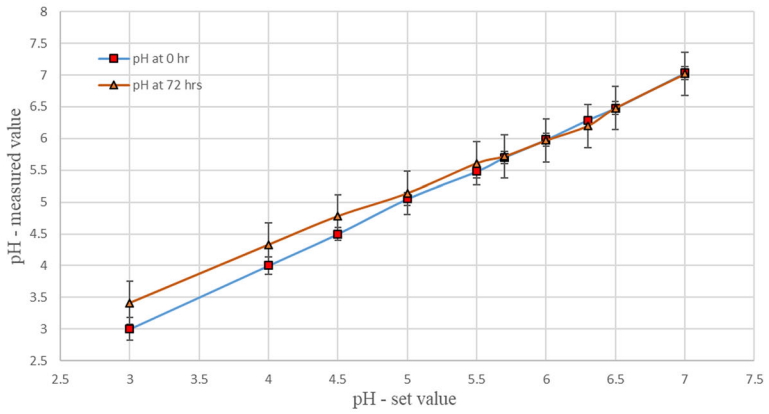

(c)

Fig. 1 pH data corresponding to time intervals from 0 h to 72 h during enzymatic hydrolysis using three different enzymes **a** Celluclast® 1.5L with Novozymes 188, **b** endo-1,4-β-xylanase, and **c** Cellic® CTec2

water to an acid concentration of 4% and autoclaved for another 60 min at 121 °C. The hydrolysis liquor was neutralized using solid calcium carbonate to pH (5.0–6.0) and centrifuged for 10 min at 4400 rpm. The supernatant was filtered by passing through a 2-μm filter paper and collected for the determination of the carbohydrates and lignin composition. High performance liquid chromatography (HPLC, Nexera-1, Shimadzu) with a UV detection

at 280 nm was used to determine the carbohydrates composition. The instrument was equipped with a Shodex sugar SP0810 column, and the separation was carried out at 80 °C. Deionized water was used as an eluent in a flow rate of 0.6 mL min^{-1} with 20 μm injected sample volume. The WS composition was 41% cellulose, 33% hemicellulose, 18% lignin, and 8% others.

Total reduced sugar yield using UV/Visible

The DNS method was used to measure the reduced sugar yield, by mixing 3 mL of DNS reagent and 1 mL of sodium citrate buffer (0.05 M) with 0.5 mL of hydrolysate supernatants. This mixture was submerged into a boiling water bath for 5 min then cooled to room temperature in a water-ice bath. 1.5 mL of sodium citrate buffer (0.05 M) and 3 mL of DNS reagent were used as a blank. All analyses were carried out in triplicate using Bibby Scientific™ 7305 Model UV/Visible Spectrophotometer at 540 nm wavelength. A calibration curve was obtained for glucose as it is the major product from WS. The calibration curve equation is $Y = 0.3098\ X + 0.0618$ with $R^2 = 0.9957$, where Y represents absorbance, and X represents the total reduced sugar concentration (1 mg/0.5 mL).

Composition analysis using GC-MS

The sugar extracted at the end of hydrolysis was centrifuged at 4400 rpm for 5 min and filtered through 0.2 μm filter paper. The samples were then evaporated to dryness, treated with 300 μL of methoxyamine hydrochloride solution in pyridine at a concentration of 20 mg mL^{-1} and incubated at 37 °C for 90 min. Aliquots equal to 300 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were added and incubated for another 60 min at the same temperature. The reduced sugar was then analyzed by gas chromatography–mass spectrometry (GC-MS) using an Agilent 6890 plus GC with a 5973 N MS, (Agilent Technologies, Palo Alto, CA, USA) equipped with a Restek column (30 m × 0.25 mm × 0.25 μm, RxI-5MS, Bellafonte, PA, USA). The GC oven temperature was kept constant for 1 min at 70 °C and gradually increased at a fixed rate of 5 °C min^{-1} until 320 °C. The injection port and transfer line temperatures were 260 °C and 280 °C, respectively. The carrier gas (helium) flow rate was 1 mL min^{-1}. The injection volume was 1.0 μL with a split injection ratio of 50:1. The data were recorded in the mass range of 50–500 m/z, and the results were specified by comparison (cross match) with standards sugars (Yang et al. 2013). The average results of duplicate runs were reported.

Results and discussions

pH evaluation before and after enzyme addition

The main objective of this study was to investigate the effect of changing the pH during the enzyme hydrolysis; therefore, pH values were measured before and after

Table 2 Influence of different particle size on total reduced sugar yield (g L^{-1}) at pH 4.8

		Total reduced sugar produced (g L−^{1})			
		24 h	48 h	72 h	94 h
Celluclast® 1.5 L + Novozymes 188	> 2000 μm	1.6 ± 0.28	3.2 ± 0.27	3.7 ± 0.28	3.7 ± 0.28
	200–1000 μm	1.8 ± 0.27	3.3 ± 0.29	3.9 ± 0.33	3.9 ± 0.33
	1000–710 μm	2.5 ± 0.29	3.6 ± 0.29	4.1 ± 0.32	4.1 ± 0.32
	710–500 μm	2.7 ± 0.31	3.8 ± 0.28	4.2 ± 0.3	4.2 ± 0.3
	500–250 μm	2.9 ± 0.25	4.1 ± 0.3	4.8 ± 0.34	4.8 ± 0.34
	< 250 μm	3.2 ± 0.3	4.8 ± 0.38	5.2 ± 0.4	5.2 ± 0.4
endo-1,4-β-xylanase	> 2000 μm	1.8 ± 0.26	3.2 ± 0.31	3.8 ± 0.25	3.8 ± 0.25
	200–1000 μm	1.9 ± 0.26	3.3 ± 0.3	3.9 ± 0.3	3.9 ± 0.3
	1000–710 μm	2.6 ± 0.28	3.7 ± 0.25	4.3 ± 0.35	4.3 ± 0.35
	710–500 μm	2.7 ± 0.33	3.9 ± 0.28	4.4 ± 0.22	4.4 ± 0.22
	500–250 μm	3.1 ± 0.32	4.2 ± 0.3	4.9 ± 0.29	4.9 ± 0.29
	< 250 μm	3.5 ± 0.3	4.9 ± 0.27	5.5 ± 0.26	5.5 ± 0.26
Cellic® CTec2	> 2000 μm	4.1 ± 0.51	5.2 ± 0.6	5.8 ± 0.54	5.8 ± 0.54
	200–1000 μm	4.3 ± 0.58	5.4 ± 0.59	6.0 ± 0.62	6.0 ± 0.62
	1000–710 μm	4.4 ± 0.62	5.9 ± 0.65	6.5 ± 0.6	6.5 ± 0.6
	710–500 μm	5.2 ± 0.62	6.7 ± 0.45	7.2 ± 0.52	7.2 ± 0.52
	500–250 μm	6.1 ± 0.56	7.5 ± 0.4	8.0 ± 0.51	8.0 ± 0.51
	< 250 μm	6.5 ± 0.64	8.5 ± 0.6	8.8 ± 0.58	8.8 ± 0.58

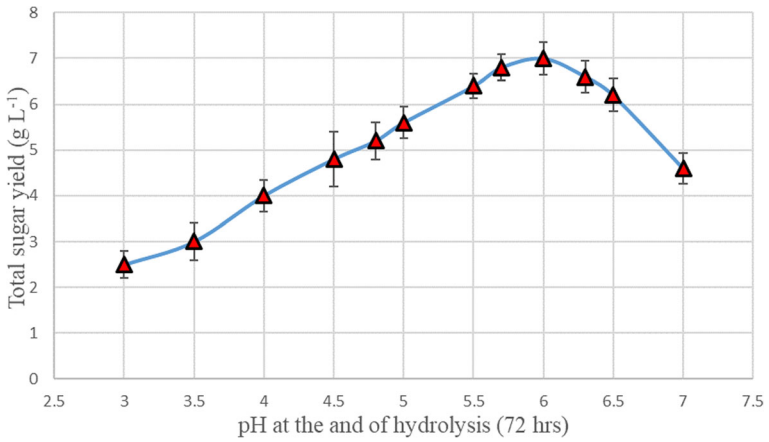

Fig. 2 Total reduced sugar concentration for different pH solution at the end of the hydrolysis (72 h)

addition of the enzymes. The measured pH values are reported in Table 1 for both before and after addition of the enzymes to the suspension (buffer solution and WS). Since the pH value increased as a result of adding the enzymes, the pH of the solutions was adjusted back to the original pH values, and this is reported as pH adjusted in Table 1. The results reported in Table 1 are the average of three replicates for each enzymes and pH value.

It was found that at low pH values, the change was higher after adding the enzymes than at high pH values due to the low acidity of the enzymes (pH 6.0–6.5). The highest increase in the pH value was noticed after adding the Ctec 2 to the pH 3 solution, with the pH value increasing from 3.0 to 3.61. While the lowest change occurs after adding the Cellic® CTec2 to the solution with pH 7.0, the increase was very low and was neglected.

pH value at the end of enzymatic hydrolysis

Due to the importance of the pH value, the pH at the start (0 h) and at the end (72 h) of the enzymatic hydrolysis are shown in Fig. 1a–c, for Celluclast® 1.5 L with Novozymes 188, endo-1,4-β-Xylanase, and Cellic® CTec2, respectively. The experiments were repeated three times with the average results presented graphically in Fig. 1.

Figure 1 a shows that for Celluclast® 1.5 l with Novozymes 188, there was a minor increase in pH at the end of the enzymatic hydrolysis. The difference in pH values was less than 0.17% at the maximum difference. On the other hand with endo-1,4-β-xylanase (Fig. 1b), there was an increase of 0.26% at the end of the enzymatic hydrolysis. A negligible change was observed after pH 4.0, and for pH 5.0–7.0, the pH value remained the same. The greatest change was seen for Cellic® CTec2 as shown in Fig. 1c. Between pH 3.0–4.8, there was an

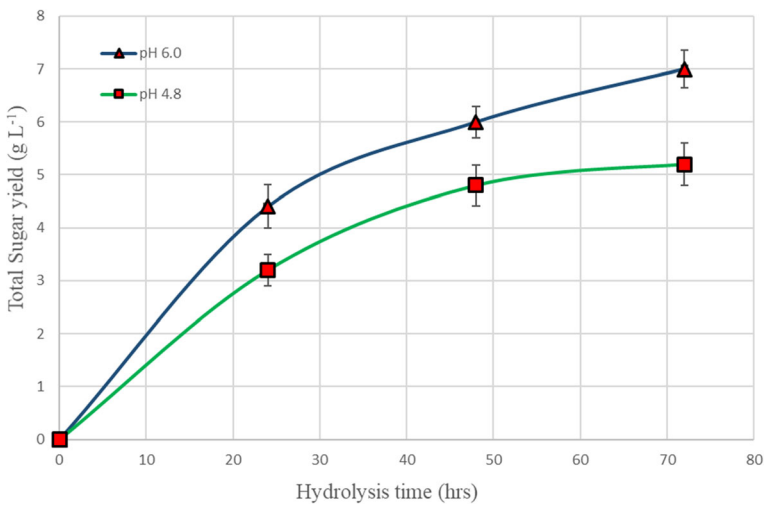

Fig. 3 Total reduced sugar yield at the end of hydrolysis (72 h) for pH 4.8 and 6.0

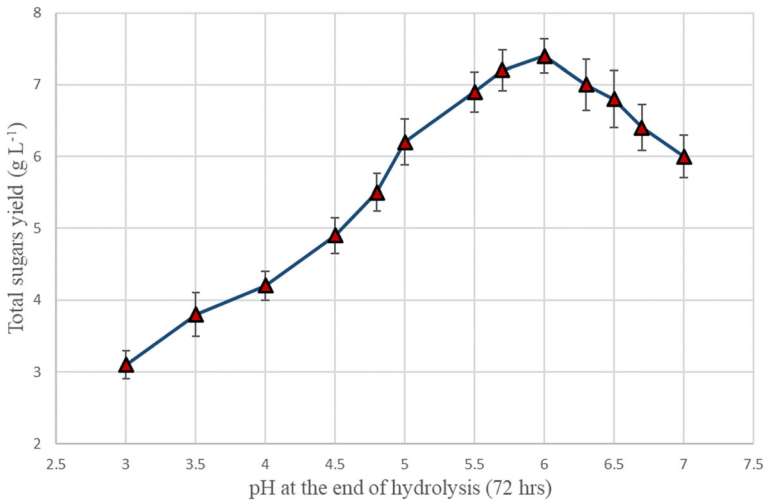

Fig. 4 Total reduced sugar concentration for different pH solution at the end of the hydrolysis (72 h)

increase in pH at the end of the enzymatic hydrolysis. The highest increase was found at pH 3.0 where the pH increased from 3.0 at (0 h) to 3.41 at (72 h). This means that there was approximately 13% increase in the pH value at the end of hydrolysis.

Although both endo-1,4-β-xylanase and Ctec 2 show the highest difference in pH value at pH 3.0 and 3.5 and pH 3.0, 3.5, 4.0, and 4.5, respectively, the difference is not very high and is within the error bar. Therefore, the adjusted pH value at (0 h) and the final pH measured at each different pH point studied show no significant difference and can be assumed to be the same.

Influence of WS particle size on reduced sugar yield

WS particle size is a fundamental parameter that affects biomass digestion efficiency. It has been suggested that grinding the WS to a fine size breaks down the lignin as well as increasing the surface area of the sample which gives the enzymes more accessibility and facilitates the biomass digestion (Hu et al. 2017).

The WS was ground using a ceramic disk and sieved to get different particle sizes ranging from less than 250 to more than 2000 μm. Then, the range of samples with different particle size was subjected to enzymatic hydrolysis at pH 4.8 using (Celluclast® 1.5 L + Novozymes 188, endo-1,4-β-xylanase, and Cellic® CTec2) for 94 h, respectively. The hydrolysis was carried out for 24 h longer than the normal 72 h to ensure reaction completion.

The reduced sugar yield was found to increase with smaller particle size as shown in Table 2. Grinding the WS to reduce the particle size increased the surface area and reduced the degree of crystallinity which gives more accessibility for enzymes and therefore increases the

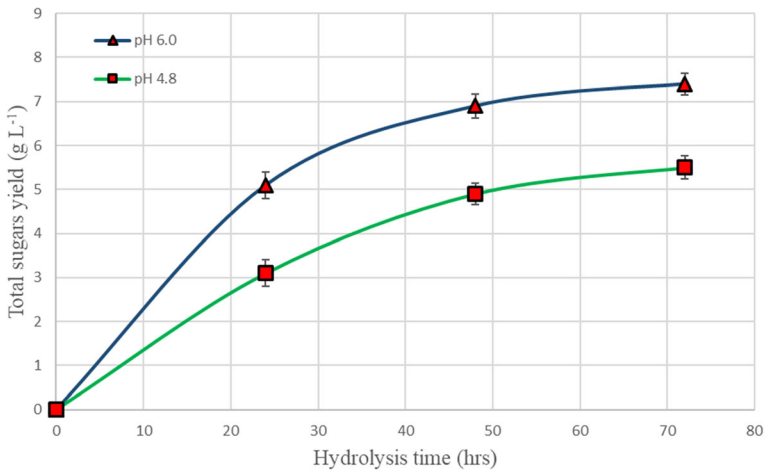

Fig. 5 Total reduced sugar yield at the end of hydrolysis (72 h) for pH 4.8 and 6.0

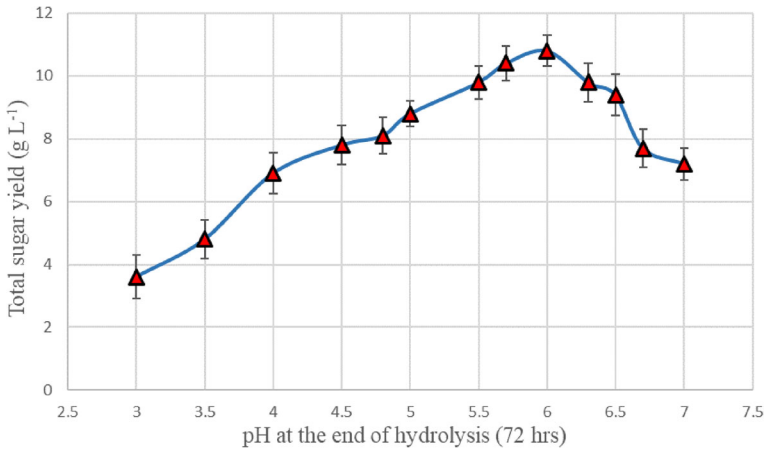

Fig. 6 Total reduced sugar concentration for different pH solution at the end of the hydrolysis (72 h)

total reduced sugar yield (Silva et al. 2012). The total reduced sugar yield increased rapidly with time up to about 50 h then it begins to level out. After 72 h, there was no significant increase in the total reduced sugar yield. It can clearly be seen that higher reduced sugar yield was obtained from the finest particle size for all the enzymes. Therefore, the sample which gave the highest reduced sugar yield (less than 250 μm) at pH 4.8 was chosen to study the pH effect on total reduced sugar yield during enzymatic hydrolysis.

Influence of pH on WS enzymatic hydrolysis using Celluclast® 1.5 L with Novozymes 188

Using the smallest particle size (less than 250 μm), 1 g of WS was enzymatically hydrolyzed using 50 mL of various pH (3.0–7.0) solutions at 50 °C and 200 rpm. Celluclast 1.5 L with Novozymes 188 was subjected to pH study since they are widely used for lignocellulosic enzymatic hydrolysis (Hu et al. 2015; Lan et al. 2013). The total reduced sugar yield was obtained using the DNS method and plotted against the pH at the end of hydrolysis (72 h) as shown in Fig. 2.

As can be seen from Fig. 2, the highest reduced sugar yield was achieved between pH 5.7 and 6.3 rather than at 4.8 as cited by most researchers (Lan et al. 2013). The total reduced sugar yield increased from 5.2 to 7.0 (g L^{-1}) by changing the pH value from 4.8 to 6.0, respectively.

To give further confirmation, the total reduced sugar yield was observed between 0 and 72 h at pH 4.8 and 6.0 and shown in Fig. 3, which clearly indicates that the total reduced sugar yield for the WS substrate increased from 5.1 to 7.1 (g L^{-1}) (approximately 28%).

Influence of pH on WS enzymatic hydrolysis using endo-1,4-β-xylanase

The experiment was repeated using the same conditions for the endo-1,4-β-xylanase enzyme as shown in Fig. 4. Similarly to the previous enzyme cocktail, pH 4.8–5.0 is currently the preferred value for enzymatic hydrolysis

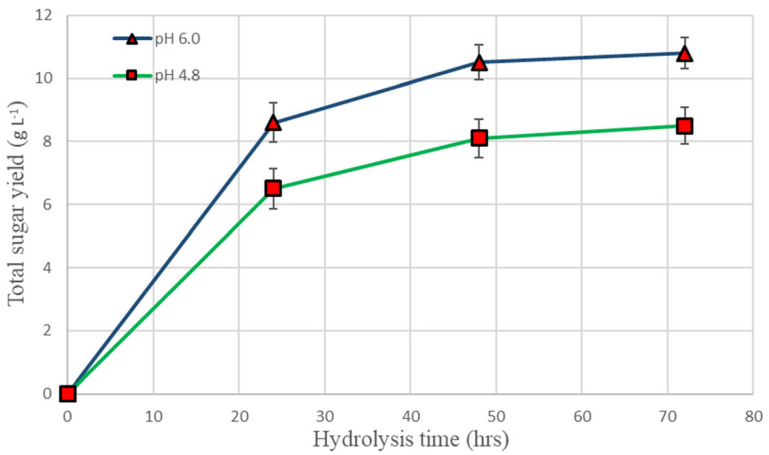

Fig. 7 Total reduced sugar yield at the end of hydrolysis (72 h) for pH 4.8 and 6.0

(Avci et al. 2013). It can be seen that there was a detectable increase in total reduced sugar yield efficiency from 3.1 to 7.4 (g L^{-1}) in the pH range of 3.0–6.0 with the optimum range being pH 5.7–6.0 instead of 4.8 as widely used by researchers.

Figure 5 shows a similar trend in the change of total reduced sugar yield with time by using endo-1,4-β-xylanase. The total reduced sugar yield increased from 5.5 to 7.4 (g L^{-1}) at pH 4.8 and 6.0, respectively.

Influence of pH on WS enzymatic hydrolysis using Cellic Cellic® CTec2

Cellulase Cellic® CTec2 is a commercial enzyme cocktail which was also subjected to the optimum pH investigation. The WS was enzymatically hydrolyzed under the same experimental conditions as for the previous enzymes (Celluclast® 1.5 L with Novozymes 188 and endo-1, 4-β-Xylanase). Figure 6 shows the total reduced sugar yield plotted against the pH value at the end of enzymatic hydrolysis (72 h).

By increasing the pH from 4.8 to 6.0, the total reduced sugar yield increased from 8.5 to 10.8 (g L–^{1}(. The total reduced sugar yield for both pH 4.8 and 6.0 was also monitored with time during the hydrolysis, and the results are shown in Fig. 7. The total reduced sugar yield from pH 4.8 and 6.0 behaves similarly with time. The gap between the reduced sugar concentration was almost constant at 2.2 (g L–^{1}) during the hydrolysis. Therefore, it is recommended to use pH 6.0 to achieve high reduced sugar yield from WS.

In summary, all the enzymes used in this study show an improvement after changing the pH.

Figure 8 illustrates the total reduced sugar yield after enzymatic hydrolysis for the enzymes at pH 4.8 and 6.0. By changing the pH of the solution from 4.8 to 6.0, Celluclast® 1.5 L with Novozymes 188 and endo-1,4-β-xylanase show an increase in the total reduced sugar yield from 5.2 to 7.0 (g L–^{1}) and 5.5 to 7.4 (g L–^{1}), respectively. In the case of Cellic Ctec 2, the total reduced sugar increased from 8.5 to 10.8 (g L–^{1}).

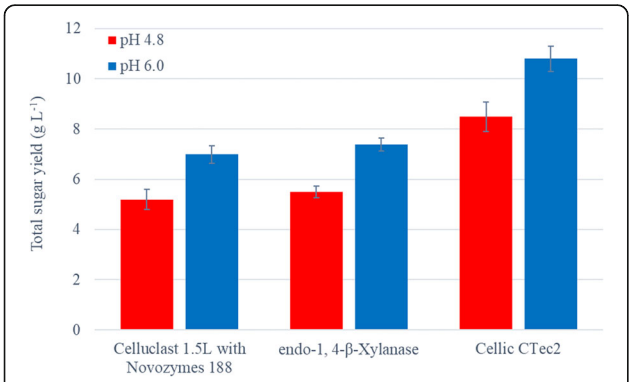

Fig. 8 Total reduced sugar yield for the enzymes at pH 4.8 and 6.0

Conclusion

The results of the present study indicate the optimum pH for enzymatic hydrolysis using different enzymes (Celluclast® 1.5 L with Novozymes 188, endo-1,4-β-xylanase, and Cellic® CTec2) is different from the range pH 4.8–5.0 used in most studies. The enzymatic hydrolysis was carried out for 94 h in the beginning; however, since there was no change in the reduced sugar yield after 72 h, there was no need to continue with enzymatic hydrolysis, and the enzymatic hydrolysis was stopped after 72 h.

The results obtained from this study indicate that the optimum pH for WS as a lignocellulosic substrate is higher than pH 4.8 which is exclusively used by almost all the existing literature. The enzymes activity test based on using pure cellulose substrate (Whatman paper) at pH 4.8 as an optimum pH suggested by cellulase manufacturers is not necessarily the same optimum value for lignocellulosic (i.e., WS) substrate.

Reducing the acidity in lignocellulosic substrate enzymatic hydrolysis might have an effect on reducing lignin inhibition of the activity of the enzyme, by reducing the lignin absorption of enzymes or affecting the lignin-cellulose binding and interaction by affecting the electrostatic charge of the lignocellulose, changing the pH could also have an effect on the lignin-derived phenols.

All the enzymes which were used in this study show a significant improvement in total reduced sugar yield after changing the pH from 4.8 to 6.0, both Celluclast® 1.5 L with Novozymes 188 and endo-1,4-β-xylanase show an increase of (25%) while Ctec 2 shows an increase of (21%).

Based on the results presented in this study, it is recommended that future work on enzymatic hydrolysis of WS as a lignocellulose substrate be conducted at a pH range of 5.8–6.0.

Nomenclature

Y Absorbance
X Concentration (mg 0.5 mL^{-1})

Abbreviations

WS: Wheat straw; FPU: Filter paper unit; DM: Dry Matter; CB: Cellobiase Unit; DNS: 3,5-Dinitrosalicylic acid; HPLC: High Performance Liquid Chromatography; GC-MS: Gas chromatography–mass spectrometry; UV: Ultraviolet; MSTFA: N-Methyl-N-(trimethylsilyl) trifluoroacetamide

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Competing interests
There are no conflicts to declare.

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