Pretreatment and enzymatic hydrolysis of agricultural residues

Kvesitadze G., Sadunishvili T., Kutateladze L., Khvedelidze R., Khokhashvili I., Urushadze T., Zakariashvili N., Tsiklauri N. And Aleksidze T.

Durmishide Institute of Biochemistry and Biotechnology, Agricultural University of Georgia

*corresponding author:

e-mail: t.sadunishvili@agruni.edu.ge

Abstract. Production of biofuels from renewable lignocellulosic materials is a good alternative to petroleum based fuels. Important route to convert biomass to biofuel is biochemical, which envisages enzymatic transformation of cellulose and hemicellulose of the wastes to glucose and microbial fermentation to the last to fuel. The goal of the project is development of cost effective biotechnology of fuel bioethanol production from agricultural residues. Different methods were applied for pretreatment of agricultural residues: wheat straw, rice straw and corn stalks. Chemical treatment of the substrates using peroxide solution 1%(w/v) H_2O_2 and adjusted to pH 11.5 with 3 N sodium hydroxide (alkaline peroxide solution) at 30°C and 60°C for 6 h has been conducted. As a result of alkaline peroxide pretreatment of lignocellulosic substrates the increase in cellulose content from 31-34% to 68-69% and corresponding decrease in lignin content from 14-19% to 4.0-4.4% is observed.The biological pretreatment - cultivation of basidial fungi strains *Pseudotrametes* sp. IK-76, *Ganoderma lucidum* GM 04, *Ganoderma applanatum* IN 59resulted in delignification from14% to 11.0% (rice straw) and from 17.3 to 14.0 (corn stalk). As a result of hydrolysis of the alkaline peroxide pretreated rice and wheat straw and corn stalk by cellulases preparations of selected *Penicillium canescence* 85, *Aspergillus* sp.Av10, *Sp.pulverulentum* T5-0 the increase in glucose yield approximately by two -three times as compared to untreated substrates is observed.

Keywords: Agricultural residues, chemical pretreatment, enzymatic hydrolysis, microscopic fungi cellulases

1. Introduction

Physical and chemical pretreatment of lignocellulosic wastes

Plant biomass a lignocellulose, is composed of three major polymers: cellulose, hemicellulose and lignin. It is built up as cellulose fibers that are partially arranged in a crystalline structure, integrated with hemicellulose and embedded in a matrix of lignin. Both cellulose and hemicellulose are the sources of fermentable sugars. Major difficulties in efficient lignocellulosic conversion and biofuel production are lignin and recalcitrance of cristalline cellulose (Gould 1984). Compared to starch and sugar crops, effective release of fermentable sugars from recalcitrant lignocellulosics requires appropriate pretreatment and hydrolysis steps.

Although various methods of cellulosic substrates pretreatment have been proposed, their effectiveness varies depending on the substrate. Thus, it is important to establish optimum pretreatment method for each substrate.

Out of chemical methods attention is paid to application of diluted alkali at different conditions such as: high pressure, boiling and treatment at room temperature (MacDonald *et al.*, 1983;Cheng *et al.*, 2010) and alkaline hydrogen peroxide treatments.

Pretreatment time has a major influence on the delignification efficiency.

Paper reports on the effeciency of hydrolysis of corn stalks, rice stalks and wheat straw, pretreated by different chemical methods.

2. Materials and methods

Agricultural residues: corn stalks, rice stalks and wheat straw were applied in studies.

Cellulose content was determined according toUpdegraff D.M. (1969). 130-160 mg of the studied substrates were soaked in acetic acid and nitric acid to remove hemicellulose and lignin. Total sugar content in solution of hydrolysed cellulose was determined by anthron test (Bailey, 1957). During heating glucose with sulphuric acid of arthron solution, colored product 5-hydroximetylfurfural is created. The concentration of 5-hydroximetylfurfural was determined spectrophoto-metrically on Jenway 6505 UV/VIS at 625 nm. The concentration of glucose was determined using standard curve was used to calculate glucose concentration which was used for calculation of the cellulose content.

Dry weight was determined by weight method according to difference between initial and final weights, by bringing sample to constant weight at 105˚C.

Hemicellulose was determined by weight method, treating sample with 0.1N H_2SO_4.

The amount of lignin was determined by treating sample with 72% H_2SO_4.

Chemical methods were applied for pretreatment of agricultural residues.

Wheat straw, rice straw and corn stalks were pretreated by sodium hydroxide at different conditions: Treatment with 10% sodium hydroxide solution in autoclave at 1 atm, 121^0C for 2 h, washing with water to neutral reaction; Treatment with 10% sodium hydroxide solution at room temperature for 24h, washing with water to neutral reaction.

Hydrogen peroxide solution 1% (w/v) $H_2O_2$was added tomechanically treated agricultural residues and the obtained suspension adjusted topH 11.5 with 3 N sodium hydroxide (alkaline hydrogen peroxide solution). Hydrogen peroxide requires an alkaline pH to produce the oxidizing radicals necessary to degrade lignin. To the mechanically treated plant substrate was added 1% H_2O_2 and the obtained suspension was adjusted to pH 11.5 as indicated in the reference paper (Gould, 1984). Treatment was performed at 30°C and 60°C for 6 h. The treated materials were washed with tap water then with distilled water until neutrality and dried at 105 °C (Yang *et al*.,2004).

Hydrolysis of sodium hydroxide pretreated agricultural residues was carried out by cellulase technical preparations ofselectedmicroscopic fungi strains of our collection: thermotolerant *Aspergillus*sp. Av10 and a mesophile*Penicillium canescence* 85 (Kvesitadze *et al*., 2016).Dried samples of chemically pretreated wheat straw was placed in separate tubes with 20 ml 0.05 M acetate buffer, pH 4.7; 10 Units of filter paper activity of the above cellulases technical preparations were added to 1 g of substrate. Such mixture was incubated at temperature regimen 50-60°C for 6, 12, 24 hours. After definite incubation time, the amount of reducing sugars and glucose were measured. Percentage of reducing sugars are calculated from the weight of substrate; Percentage of glucose, from weight of sugars produced.

Hydrolysis of alkaline hydroxide peroxide solution treated cellulosic substrates were performed by technical preparations of *Aspergillus*sp. Av10,*Penicillium canescence* 85 and *Sp.pulverulentum* T5-0 in the same above described conditions.

3. Results and discussion

The effect of different chemical treatments of wheat straw, corn and rice stalks is evaluated according to change of chemical composition of the agricultural residues Results are represented in Tables1 and 2.

Table 1. Composition of NaOH and high pressure treated wheat and rice straw, and corn stalks (Data is expressed as percentage content on a substrate dry weight basis)

Substrates	Pretreatment	Chemical components,%			
		Hemicellulose	Cellulose	Lignin	Ash
Wheat straw	10% NaOH, 1 atm .pressure (121°C), 2h	17.5	39,1	7.0	3.2
	10% NaOH at room temp. 24h	20.0	34.1	10.0	4.4
	Untreated	26.0	32.0	19.0	10.5
Corn stalk	10% NaOH, 1 athigh-pressure (121°C), 2h	20.6	40.5	9.5	2.2
	10% NaOHAt room temp. 24h	25.5	37.8	12.5	3.6
	Untreated	30.0	31.2	17.3	9.2
Rice straw	10% NaOH, 1 athigh-pressure (121°C), 2h	20.5	38.8	8.5	3.6
	10% NaOHAt room temp. 24h	23.6	35.6	10.2	4.8
	Untreated	29.1	34.5	14.1	12.0

Table 2. Composition of alkaline hydroxide treated wheat and rice straw, and corn stalks (Data is expressed as percentage content on a substrate dry weight basis)

Substrates	Pretreatment by 1% H_2O_2 pH 11.0, 6 h (AHP)	Chemical components,%			
		Hemicellulose	Cellulose	Lignin	Ash
Wheat straw	AHP at 30^0C	23.1	66.0	6.0	4.9
	AHP at 60^0C	22.2	68.8	5.0	4.0
	Untreated	26.0	32.0	19.0	10.5
Corn stalk	AHP at 30^0C	26.1	67.2	4.4	2.3
	AHP at 60^0C	27.5	66.4	4,0	2.1
	Untreated	30.0	31.2	17.3	9.2
Rice straw	AHP at 30^0C	24.1	68.2	4.4	3.3
	AHP at 60^0C	23.5	69.4	4.0	3.1
	Untreated	29.1	34.5	14.1	12.0

Table 3. Hydrolysis parameters of the 10% NaOH pretreated and autoclaved wheat strawby cellulases obtained from microscopic fungi strains

Origin of Cellulases	Hydrolysis duration, h	Yield of Reducible sugars (RS), %	Yield of glucose, %
Aspergillus sp. Av10 (thermotollerant)	6	15	25
	12	23	35
	24	45	43
Penicillium canescence 85 (mesophile)	6	11	18
	12	32	34
	24	42	37

Table 4. Hydrolysis parameters of the 10% NaOH pretreated wheat straw by cellulases obtained from microscopic fungi strains

Origin of Cellulases	Hydrolysis duration, h	Yield of Reducible sugars (RS), %	Yield of glucose, %
Aspergillus sp. Av10 (thermotollerant)	6	12	15
	12	20	28
	24	40	35
Penicillium canescence 85 (mesophile)	6	10	11
	12	28	30
	24	38	32

Table 5. Hydrolysis parameters of the AHP pretreated agricultural residues by cellulases obtained from microscopic fungi strains

Substrates	Pretreatment by 1% H_2O_2 pH 11.0, 6 h (AHP)	Cellulases		
		P.canescens 85	*Aspergillus* sp.Av 10	*Sp.pulverulentum* T5-0

		Yield of reducible sugars(RS) and Glucose (G)					
		RS,%	G,%	RS,%	G,%	RS,%	G,%
Wheat straw	AHP at 30^0C	72.0	80.0	70.0	85.0	75.0	88.0
	AHP at 60^0C	70.0	81.0	70.0	82.0	76.0	87.0
	Untreated	40.0	35.0	42.0	40.0	38.0	45.0
Corn stalk	AHP at 30^0C	82.0	87.0	78.0	80.0	76.0	80.0
	AHP at 60^0C	81.0	86.0	78.0	81.0	75.0	82.0
	Untreated	34,0	38.0	38.0	42.0	32.2	42.0
Rice straw	AHP at 30^0C	90.0	98.0	87.0	80.0	95.0	95.0
	AHP at 60^0C	88.0	94.0	85.0	80.0	95.0	94.0
	Untreated	45.0	46.0	14.1	12.0	45.0	51.0

As seen as a result of sodium hydroxide treatment the amount of cellulose is increased with corresponding decrease of lignin content. Comparatively better results were obtained in case of lignocellulosic substrates pretreatment with 10% NaOH and high pressure, with the best delignification in case of wheat straw. The delignification of wheat straw biomass was significant even at room temperature treatment (Table 1).

Alkaline hydrogen peroxide (AHP) treatment appeared more effective as compared to sodium hydroxide treatment. AHP treatment resulted in almost 2 times increase in cellulose content and significant delignification. The most effective delignification (77%) is achieved for wheat strawat 60^0C. It should be stressed that the AHP treatment results in effective delignification of the studied substrates at both tested temperatures 30^0C and 60 ^{0}C with minimum difference.

Enzymatic hydrolyses of chemically pretreated by the above described two methods agricultural residues were studied (Khvedelidze *et al*., 2016).

As seen from the Tables 3 and 4, as a result of exhaustive (24-hour) hydrolysis of the NaOH pretreated wheat straw 42-45% were hydrolyzed to reducing sugars, out of which 37-43% is glucose.

Alkaline peroxide treatment appeared more effective and resulted in significant increase in hydrolysing products (Table 5). For example, yields of glucose in AHP treated rice stalks by the thermotolerant *A.awamori* cellulase increased from 12% to 80%.

4. Conclusions

10% NaOH and alkaline peroxide treatments of mechanically grinded agricultural residues resulted in significant delignification of the lignocellulosic substrates. AHP appeared more effective, according to high glucose yield as a result of enzymatic hydrolyses of AHP pretreated substrates.

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