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Enzymatic Hydrolysis of Ball-Milled Saudi Wheat Straw

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ABSTRACT. Pretreated Saudi wheat straw was enzymatically hydrolyzed using cellulase [EC 3.2.1.4] in citric acid and sodium hydroxide buffer solution at pH value of 5.4. Decreasing the wheat straw particle size and/or the rotation speed of the mixer was found to increase the concentration of the produced glucose. Also, increasing the cellulase to wheat straw ratio causes an increase in the yield of glucose and an asymptotic value was reached at about ten hours of reaction time. The yield of glucose was found to go through a maximum upon increasing the reaction temperature from 30 to 55°C indicating an optimum temperature. This optimum glucose yield was found to occur at 37°C.

KEY WORDS: Cellulose, Enzymatic hydrolysis, Cellulose pretreatment.

1. Introduction

Wheat is the major agricultural product in Saudi Arabia. It is produced in large quantities sufficient to meet local consumption needs as well as exportation to other countries of the world.

Large amounts of wheat straw are left behind after harvesting of wheat. The estimated amount of wheat straw generated in Saudi Arabia in 1987 is about 3.65×10^6 tons[1]. Part of the straw is used as animal fodder while the remainder is disposed off as a solid waste.

Analysis of Saudi wheat straw[1] has shown that it contains an average of 31.6%, 34.0%, 22.3% and 12.1% of α-cellulose, hemicellulose, lignin and ash respectively. α-cellulose is a valuable substance. It can be hydrolyzed to produced glucose which can, further, be isomerized to fructose or fermented to ethanol. It, also, can be converted into pulp to make appear, tissues, ... etc.

Hydrolysis of wheat straw can be carried out by acid processes or by enzymatic processes[2-7]. Acid hydrolysis is usually performed by using concentrated or dilute acids. Both processes suffer from difficulties in recovering the acid, sugar decomposition, and corrosion problems. On the other hand, enzymatic hydrolysis is very specific and as such no side reactions or sugar decomposition are observed. However, enzymatic reaction is rather slow.

Native cellulose has high degree of polymerization and is mostly crystalline. These factors together with the presence of lignin are responsible for the slowness of the enzymatic hydrolysis. Generally in hydrolysis, the hydrolytic reagent has to reach and break the oxygen bridge between two glucose units in the cellulose polymer. Therefore, very often enzymatic processes require pretreatment of the substrate to avail the oxygen bridge. Several pretreatment techniques have been used to increase the susceptibility of cellulose to enzymatic hydrolysis. Among these are: Size reduction, ß-irradiation, chemical treatment, and hydrothermal methods[8,9].

The objective of this study is to investigate the effect of changing particle size, enzyme loading, temperature, and agitation on enzymatic hydrolysis of Saudi wheat straw.

2. Experimental

2.1 Chemicals and Materials

Representative sample of wheat straw obtained from Riyadh region was used in this study. It contained, on average, 31.6% cellulose. The sample was washed to remove any attached earthly dirt and then dried. Wheat straw was pretreated by using a ball mill equipped with ceramic balls. Finally, the ground material was seived. The wheat straw retained in each tray was kept in a closed bottle and used later in experiments. 1, 4-ß-Glucan-4-Glucanohydrolase from Trichoderma Viride (BDH Co.) is used as cellulase. Buffer solutions containing citric acid and sodium hydroxide were used to adjust the pH of the hydrolysis at 5.4. All other chemicals used were Analar grade.

2.2 Experimental Method

Hydrolysis reaction was performed in 500 ml Erlenmeyer flasks. The working volume was 250 ml of which 25 ml are buffer solution. Lauda MS/2 water bath equipped with a temperature controller was used to control the reaction temperature at ± 0.1°C of the set point. Table 1 presents the components needed to prepare each of the hydrolysis solutions.

Four sets of experiments were performed in this analysis. The general strategy followed in this investigation was to determine the effect of changing particle size, rotation speed, enzyme loading and reaction temperature. Table 2 contains a summary of the numerical values of parameters undertaken in this study. Reduced sugars were determined following the method of Nelson[10]. Bausch & Lomb Spectronic 20 at wave length of 540 nm was used. The transmittence was correlated to the sugar concentration via a calibration curve.

TABLE 1. Components used to prepare the hydrolysis solution.

Components	Amount
Wheat straw	1.25 g
Cellulase	Table (2)
Buffer type	citric acid and sodium hydroxide
Volume of buffer	25 ml
Working volume	250 ml
pH	5.4

TABLE 2. Numerical values of the parameters studied.

Parameters studied	Run #	Particle size* (mm)	Rotation (rpm)	Enzyme to straw ratio (EU/g)#	Temperature °C
Particle size	1	0.125	125	16.0	37
	2	0.625	125	16.0	37
	3	1.500	125	16.0	37
Mixer rotation	1	0.125	125	16.0	37
	2	0.125	133	16.0	37
	3	1.125	148	16.0	37
Cellulase to wheat straw ratio	1	0.125	125	32.0	37
	2	0.125	125	48.0	37
	3	0.125	125	64.0	37
Temperature	1	0.125	125	64.0	30
	2	0.125	125	64.0	45
	3	0.125	125	64.0	55

*Indicates average particle size, *e.g.*, 0.125 mm represents fraction that passed through 0.25 mm mesh but retained by pan.
EU = Enzyme unit.

3. Results and Discussion

The results of the four sets of experiments performed is shown in Fig. 1-4. Figure 1 is a plot of the glucose concentration against reaction time at three different particle sizes. It is clear from the figure that as the wheat straw particle size decreases, the

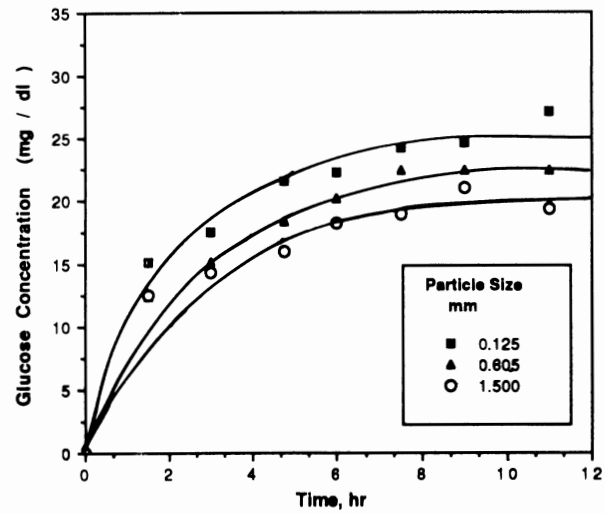

FIG. 1. Effect of particle size on hydrolysis of Saudi wheat straw (T = 37°C, pH = 5.4, enzyme loading = 16 EU/g and RPM = 125).

concentration of glucose increases. This is true since upon decreasing the particle size more surface area of wheat straw is exposed to enzymatic action.

The effect of stirring is shown in Fig. 2. It is evident from the figure that as the speed of mixer rotation is increased, the glucose concentration decreases. This is due to the fact that at relatively low speeds of rotation sufficient contact time between the substrate and the enzyme is available. However, as the speed is increased the contact time decreases thus resulting in lower glucose concentrations.

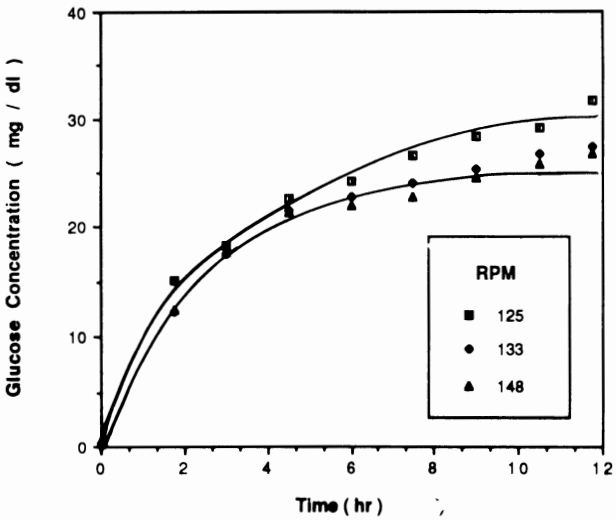

FIG. 2. Effect of mixer rotation on enzymatic hydrolysis of Saudi wheat straw. (T = 37°C, pH = 5.4, enzyme loading = 16 EU/g, and particle size = 0.125 mm).

In the next two sets of experiments, longer hydrolysis periods are used. The effect of cellulase to wheat straw ratio (enzyme loading) is depicted in Fig. 3. Increasing this ratio was found to increase the glucose concentration. Clear asymptotic values were observed as the hydrolysis time was increased. Table 3 shows that the glucose yield increases with increasing enzyme loading.

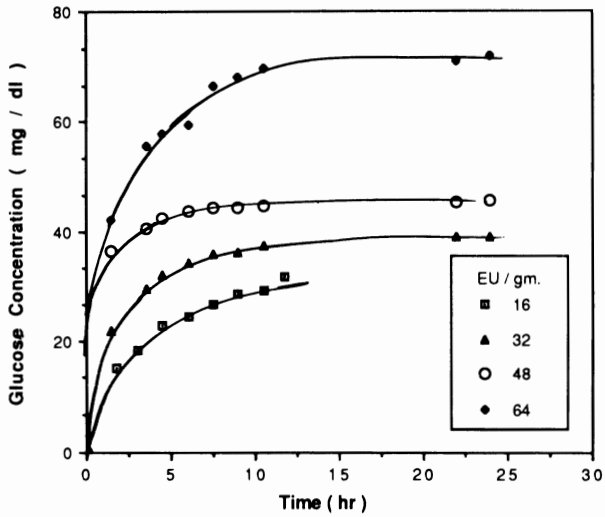

FIG. 3. Effect of enzyme loading on enzymatic hydrolysis of Saudi wheat straw. (T = 37°C, pH = 5.4, RPM = 125, and particle size = 0.125 mm.)

The effect of reaction temperature on enzymatic hydrolysis of wheat straw is illustrated in Fig. 4. The figure shows that in the range of investigation, *i.e.*, 30°C to 55°C, a maximum of glucose concentration occurs at 37°C. For clarity purposes data form the same set are plotted in Fig. 5. In Table 4, the glucose yield at various reaction times is shown against reaction temperature. Focusing on the yield at 37°C and above, one could easily see that the yield decreases upon increasing the hydrolysis temperature. This is probably due to enzyme deactivation at relatively high temperature.

4. Conclusion

Hydrolysis of representative sample of Riyadh region, Saudi Arabia, wheat straw was carried out by using cellulase from *Trichoderma viride*. Grinding was used as a pretreatment technique for the wheat straw. The results indicate that smaller size particles are more susceptible to enzymatic hydrolysis. Decreasing the agitation was found to increase the glucose yield. The glucose yield increases with increasing the enzyme loading. The optimum temperature at which maximum glucose yield occurs was determined to be 37°C. A plausible reason for the above results is that grinding availed more surface area of wheat straw to enzymatic attack. Also, at moderately low agitation speed, enough contact time between the cellulase and substrate is provided resulting in higher glucose yields. The optimum in the temperature is probably due to the fact that enzyme deactivation occurs at high reaction temperature.

TABLE 3. Variation of glucose yield with enzyme loading.

Time (hr)	16 (EU/g)	24 (EU/g)	32 (EU/g)	64 (EU/g)
0.00	0.000	0.000	0.000	0.000
1.50	–	0.138	0.230	0.267
1.75	0.096	–	–	–
3.00	0.116	–	–	–
3.50	–	0.185	0.257	0.352
4.50	0.143	0.202	0.268	0.366
6.00	0.153	0.215	0.276	0.376
7.50	0.167	0.226	0.280	0.420
9.00	0.179	0.227	0.281	0.431
10.50	0.184	0.236	0.282	0.440
11.75	0.200	–	–	–
22.00	–	0.247	0.286	0.449
22.17	0.200	–	–	–
24.00	0.200	0.247	0.288	0.455

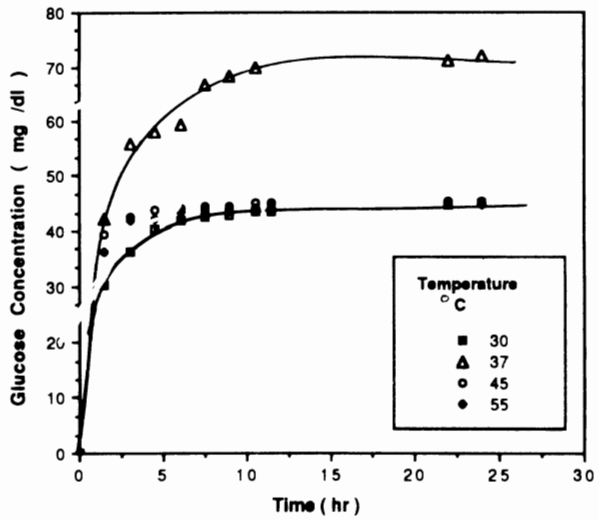

FIG. 4. Effect of reaction temperature on enzymatic hydrolysis of Saudi wheat straw. (pH = 5.4, enzyme loading = 64 EU/g, particle size = 0.125 mm and RPM = 125).

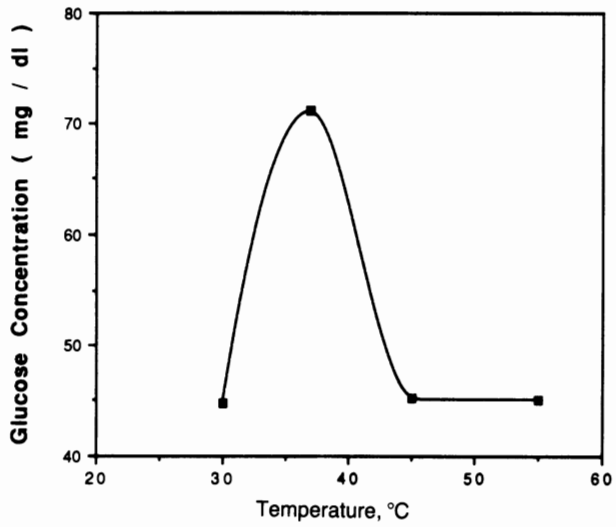

FIG. 5. Optimum temperature for enzymatic hydrolysis of Saudi wheat straw. (pH = 5.4, enzyme loading = 64 EU/g, particle size = 0.125 mm and RPM = 125).

TABLE 4. Variation of glucose yield with temperature.

Time (hr)	30°C	37°C	45°C	55°C
0.00	0.000	0.000	0.000	0.000
1.50	0.189	0.267	0.228	0.248
3.00	0.228	0.352	0.264	0.269
4.50	0.254	0.366	0.266	0.277
6.00	0.265	0.376	0.274	0.278
7.50	0.269	0.421	0.276	0.280
9.00	0.271	0.431	0.278	0.280
10.50	0.274	0.440	0.278	0.284
11.50	0.274	–	0.282	0.285
22.00	0.282	0.449	0.284	0.286
24.00	0.284	0.455	0.283	0.286

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التحلـــيل المـــائي الإنـزيمي لقـش القمـــح السعـودي المطحون بالكرات

أنيس حمزة فقيها ، أحمد الحاج أباسعيد و عصام جمال
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المستخلص . تم تحليل قش القمح السعودي المطحون باستخدام الكرات مائيًا باستخدام إنزيم السلوليز [EC3.2.1.4] في وجود محلول من حامض الستريك والصودا الكاوية ، للحفاظ على الأس الهيدروجيني عند رقم ٤,٥ . وقد وجد أن النقص في حجم قطع قش القمح و/أو زيادة سرعة التقليب تزيد من تركيز الجلوكوز المنتج . كما أن زيادة نسبة السلوليز إلى قش القمح تؤدى إلى زيادة إنتاجية الجلوكوز ، حتى تصل إلى قيمة ثابتة بعد عشر ساعات من بدء التفاعل . وقد لوحظ أن إنتاجية الجلوكوز تصل إلى قيمة عليا في نطاق درجة حرارة تتراوح بين ٣٠ إلى ٥٥°م ، وأن أقصى إنتاج للجلوكوز يحدث عند درجة حرارة ٣٧°م .