

PRODUCTION OF ETHANOL FROM AGRO-INDUSTRIAL WASTES: I. PRETREATMENT OF RAW MATERIALS FOR USING IN FERMENTATION PROCESSING.

Ammar, A. K. and S. Y. Elsanat

Food Technol. Dept., Fac. Agric., Kafr El-Sheikh Univ., Egypt.

ABSTRACT

This study aimed to utilize of the agro-industrial wastes as a cheap materials for the production raw material for using in fermentation processing, at the same time eliminates environmental impact of open burning of waste. Conditions for acid hydrolysis of waste, to produce hydrolysate containing invert sugar were study. In this respect, milled rice straw, cotton stalks and beet pulp were subjected to pretreatment using diluted acid solution at high temperature to enable production of the maximum amount of fermentable sugars from acid hydrolysis. The effects of various operating variables including pretreatment temperature, pretreatment time of reaction, the concentration of acid, the solid-to-liquid ratio and soaking time on the degree of lignin removal and liberation of reducing sugar. Acid pretreatment was carried out with H₂SO₄ at 1–5% (w/v) concentrations at 121°C and Solid: liquid ratio ranged from 1:5 to 1:25(W/V) for time ranging from 20 to 120 min. The results reveal that, there was a significant differences among the three by-products. The optimal reaction conditions, which resulted in an acid hydrolysis, were (1:20) (w/v) solid to liquid ratio; 121°C hydrolysis temperature; 4% (w/v) acid concentration, 90min reaction time and pre-soaked for 36 hours.

Keywords: Ethanol; rice straw; cotton stalk; beet pulp; acid hydrolysis.

INTRODUCTION

Ethanol is viewed as a potential fuel that is available from biomass and hence new methods to generate ethanol from hitherto inaccessible sources are gaining importance. Biotechnology for efficient utilization of lignocelluloses wastes as fuels relies on the utilization of both the cellulose as well as hemicellulosic portions of the biomass (Lachke, 2002).

Agricultural residues such as wheat straw, corn stover, rice straw and cotton stalk are a renewable resources that store energy from sunlight in its chemical bonds. It can be processed either chemically or biologically by breaking the chemical bonds to extract energy in the form of biofuels such as bioethanol, biodiesel and methane (McKendry, 2002, Diep *et al.*, 2012). Sugar beet pulp, a lignocellulosic by-product of the sugar industry, is a well-known potential source of cellulose as the lignocellulosic fraction of dried sugar beet pulp contains 22-30 % cellulose, which, have strong potential for a number of applications (Toğrul and Arslan, 2003).

Currently, molasses is the primary raw material for ethanol production in Egypt. The production of ethanol from molasses or starch impacts negatively on the economics of the process, thus making ethanol more expensive compared with fossil fuels. Lignocellulosic feedstocks, which have the potential to reduce the cost of producing ethanol because they are less expensive than molasses and available in large quantities, offer a

plausible alternative. One promising technology is to convert this abundant and renewable lignocellulosic biomass to ethanol through an enzyme-based process (Schell *et al.*, 2003 and Silverstein *et al.*, 2007).

The conversion of lignocellulosic biomass to ethanol is difficult, due to the complex structure of the plant cell wall. Pretreatment is required to alter the structural and chemical composition of lignocellulosic biomass to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars (Chang and Holtzapfle, 2000). There are mainly two process involved in the conversion: hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugar. The sugar mixture may contain any combination of xylose, arabinose, glucose, galactose, manose, fucose and rhamnose depending on the source (Karimi *et al.*, 2006a and Silverstein *et al.*, 2007).

Although traditional *Saccharomyces cereveciae* ferment glucose to ethanol rapidly and efficiently, it cannot ferment other sugar such as xylose and arabinose to ethanol (Karime *et al.*, 2006b; Shia *et al.*, 2009 and Vućurović *et al.*, 2012).

Field burning is the major practice for removing agricultural residues, but it increases the air pollution and consequently affects the public health. Therefore, to fully utilize agricultural wastes such as (rice straw, cotton stalk and beet pulp) as a feedstock for ethanol production, optimal pretreatment is required to render the cellulose fibers more amenable to the action of hydrolytic enzymes. This study was initiated to investigate the effect of sulfuric acid of rice straw, cotton stalk and beet pulp. In addition, the pretreatment (s) were identified which provide the highest cellulose to conversion to glucose during subsequent acid hydrolysis.

MATERIALS AND METHODS

MATERIALS:

Rice straw and cotton stalk materials were obtained from obtained from Sakha Research Station, Agriculture Research Center, Sakha, Kafr El-Sheikh, Egypt, during the summer season of 2007. While beet pulp was obtained from Delta Sugar Company located in Elhamol city, Kafr El-Sheikh government, Egypt.

Methods:

Preparation of raw material:

The whole waste was dried at 80°C to a constant weight then was milled in kitchen blender to give powder (80 mesh), which was used for further investigation.

Gross chemical composition (moisture, protein, ether extract and ash content) determined according to the methods described in A.O.A.C. (2000). Cellulose, hemicellulose and lignin were determined according to Sun *et al.* (1996). Reducing sugars were estimated by 3,5 dinitrosalicylic acid method (DNS), according to (Miller, 1959).

Acid hydrolysis:

Waste powder was mixed with H₂SO₄ acid to desired final concentration of (1, 2, 3, 4 and 5% w/v) in 250 Erlenmeyer flask with desired

solid to liquid ratio of (1:5, 1:10, 1:15, 1:20 and 1:25 w/v). The waste powder was soaking for different times (12, 24, 36 and 48 hours). Hydrolysis was performed at 121°C in an autoclave for variable time (20, 30, 60, 90 and 120 min.). The reaction began when the slurries of waste reached the desired reaction temperature, after the reaction was completed, the remaining solid was separated by filtration. The filtrate was then neutralized (pH =7) using 2N NaOH solution, and examined for reducing sugar.

Statistical analysis:

The data were analyzed according to (Steel and Torrie, 1980). A one way analysis of analysis of variance (ANOVA) using the general linear models (GLM) procedure was used to test for main effects where more than two variables being compared. Differences with P values <0.01 were considered statistically significant.

RESULTS AND DISCUSSION

Chemical analysis of wastes:

Lignocellulosic biomass is a very heterogeneous and chemically complex renewable resource. Rice straw, cotton stalk and beet pulp were chemically analyzed for its content of moisture; protein; ether extract; ash and lignocellulosic components (lignin, cellulose and hemicellulose).

Table (1): General chemical composition of rice straw, cotton stalk and beet pulp as agro-industrial residues and wastes (on dry weight basis)*.

| Components (%) | Rice straw | Cotton stalk | Beet pulp |
|----------------|--------------------|--------------------|---------------------|
| Moisture | 10.12 ^a | 8.50 ^b | 10.00 ^a |
| Dry matter | 89.88 ^b | 91.50 ^a | 90.00 ^{ab} |
| Crude protein | 2.36 ^c | 3.22 ^{bc} | 8.95 ^a |
| Ash content | 15.11 ^a | 6.17 ^b | 3.74 ^c |
| Ether extract | 0.98 ^b | 0.75 ^c | 1.15 ^a |
| Holocellulose | 62.99 ^b | 72.86 ^a | 56.85 ^c |
| α- Cellulose | 36.14 ^b | 58.48 ^a | 27.50 ^c |
| Hemicellulose | 26.85 ^b | 14.38 ^c | 29.35 ^a |
| Lignin | 13.10 ^b | 21.45 ^a | 3.82 ^c |

*Each value was average of three determinations.

A, b and c: comparison of means of component % by type by-product.

Means within a row not sharing superscript are significantly different (P <0.01).

The previous lignocellulosic materials have the potential to be converted to bioethanol especially due to their high cellulose content. Additionally, cotton stalks contain relatively less ash than other non wood fiber resources. The results of chemical analysis of studied agricultural wastes presented in Table (1) indicate that, more than 60% of the dry matter is cellulose that is easily converted to fermentable sugars and used as cheap carbon source for many microbial industries. Wastes also contains nitrogen and ash, thereby its hydrolysate might be suitable for using as a fermentation medium. The data showed that for different lignocellulosic wastes amount of cellulose in range between 27.50 and 36.14%. These finding are in

accordance with those obtained by Howard *et al.* (2003); Shia *et al.* (2009); Vučurović *et al.*, 2012 and Kaura *et al.* (2012), who found that the cellulose content of lignocellulosic wastes of cotton stalks was ranged between 32.1 and 35.0% (on dry weight basis).

Effect of acid concentration on the liberation of reducing sugars

The effects of different acid concentrations (1-5% w/v) on hydrolysis of rice straw, cotton stalk and beet pulp wastes at 1:10 (solid to liquid ratio) at 121°C were investigate. The results in Table (2) show that at acid concentrations from 1-4 % (w/v), the release of reducing sugars during the hydrolysis increased with elongation the hydrolysis time and elevating the concentration of the acid. The highest amount of sugars liberated was 36.48, 26.55 and 36.32 (g/l) for rice straw, cotton stalk and beet pulp, respectively. This yield was obtained with acid concentration of 4% (w/v) at 121°C for 20 min. After that, a decrease in reducing sugars was observed, probably due to either repolymerization or degraded to by-products such as hydroxymethyl furfural (Prieto *et al.*, 1986 and Korish & Elsanat, 2007).

Table (2): Effect of acid concentration (H₂SO₄%) on the formation of reducing sugars.

| Acid Conc. (%) | Hydrolytic treatment | | | | Lignocellulosic materials | | | | | |
|----------------|----------------------|-------------------|-------------------|--------------------|---------------------------|--------|---------------------------|--------|------------------------|--------|
| | Solid:liquid (W/V) | Soak. time (hour) | React. temp. (°C) | React. time (min.) | Rice straw ^b | | Cotton stalk ^a | | Beet pulp ^c | |
| | | | | | RS (g/L) | SR (%) | RS (g/L) | SR (%) | RS (g/L) | SR (%) |
| 1 ^E | 1 : 10 | 12 | 121 | 20 | 23.83 | 79.2 | 14.04 | 62.5 | 23.36 | 61.9 |
| 2 ^D | | | | | 24.37 | 69.5 | 18.19 | 61.7 | 23.99 | 59.2 |
| 3 ^C | | | | | 25.59 | 65.5 | 20.25 | 60.6 | 25.33 | 57.9 |
| 4 ^A | | | | | 36.48 | 57.3 | 26.55 | 60.4 | 36.32 | 56.9 |
| 5 ^B | | | | | 25.95 | 54.5 | 26.51 | 59.1 | 25.59 | 55.5 |

*Each value was average of three determinations.

RS = reducing sugar and SR = solid recovered

a, b and c: comparison of means of reducing sugar % by type of waste.

A, B, C, D and E: comparison of means of yield % by acid concentration %.

Means within a column not sharing superscript are significantly different (P <0.01).

Effect of solid to liquid ratio on the liberation of reducing sugars:

To verify the best concentration of waste powder, which liberates the highest sugar amount during the acid hydrolysis, different solid to liquid ratios varied from 1:5 to 1:25 (w/v) were subjected to hydrolysis. The effect of solid/liquid ratio of acid on reducing sugar content of rice straw, cotton stalk and beet pulp are given in Table (3). The results presented in this Table indicate that in general the liberation rates of reducing sugars decreased by elevating the solid to liquid ratios. The formation of hydroxymethyl furfural (HMF), the dehydration product of glucose, increased with increasing of both the solid to liquid ratio. This could be attributed to the heat effect, since it did not distribute well at high solid to liquid ratio causing sugar decomposition. Similar results were reported by Kim and Hamdy (1985) and Korish and Elsanat (2007)..

Table (3) Effect of Solid : liquid ratio (w/v) on the formation of reducing sugars.

| Hydrolytic treatment | | | | | Lignocellulosic materials | | | | | |
|----------------------|---------------------|-------------------|-------------------|--------------------|---------------------------|--------|---------------------------|--------|------------------------|--------|
| Acid conc. (%) | Solid: liquid (W/V) | Soak. time (hour) | React. temp. (°C) | React. time (min.) | Rice straw ^b | | Cotton stalk ^c | | Beet pulp ^a | |
| | | | | | RS (g/L) | SR (%) | RS (g/L) | SR (%) | RS (g/L) | SR (%) |
| 4 | 1 : 5 ^E | 12 | 121 | 20 | 6.98 | 64.9 | 5.38 | 68.8 | 16.93 | 59.3 |
| | 1 : 10 ^D | | | | 36.48 | 57.3 | 26.55 | 60.4 | 36.32 | 56.9 |
| | 1 : 15 ^C | | | | 39.19 | 61.8 | 31.84 | 61.8 | 42.66 | 58.7 |
| | 1 : 20 ^A | | | | 52.08 | 61.4 | 33.56 | 64.4 | 54.56 | 62.4 |
| | 1 : 25 ^B | | | | 51.99 | 64.7 | 31.79 | 61.1 | 52.40 | 62.5 |

RS = reducing sugar

SR = solid recovered (g/ 100 g sample)

a, b and c: comparison of means of reducing sugar % by type of waste.

A, B, C, D and E: comparison of means of yield % by Solid: liquid (W/V).

Means within a column not sharing superscript are significantly different (P <0.01).

The data indicate that the most effective solid/ liquid ratio was 1:20 which the highest amount of reducing sugars liberated was 52.08, 33.56 and 54.56 (g/l) for rice straw, cotton stalk and beet pulp, respectively. Data of ANOVA given in Table (3) show that there was a significant difference at (P<0.01) among treatments

Effect of reaction time on the liberation of reducing sugars

Effect of reaction time on liberation of reducing sugars from rice straw, cotton stalk and beet pulp as affected by varying the hydrolysis time between 30 and 120 minutes, using the selected optimum conditions of acid concentration (4%), solid: liquid ratio (1:20) (W/V) and temperature (121°C) are given in Table (4). The results from this table show that the most effective reaction time was 90 minutes. At this conditions the amount of reducing sugars liberated was 36.63%, 21.76 and 41.53% for rice straw, cotton stalk and beet pulp, respectively. On the other hand, increasing the hydrolysis time to 120 min. showed no significances for all components of the hydrolysate. A fact that 90 min. of acid hydrolysis could be established as an optimum time for the hydrolysis of rice straw, cotton stalk and beet pulp to obtain a reducing sugar suitable to be used for example to produce ethanol at concentrations that varied according to the nature of the lignocellulosic material and the hydrolysis conditions. These results are in disagreement with those of Sun *et al.* (2002), they found that the optimum temperature and time for cellulose isolation were 120°C for 20 minutes. (Kim & Hamdy, 1985 and Sun and Hughes, 1998 reported similar results). Data of ANOVA given in Table (4) show that there was a significant difference at (P < 0.01) among treatments.

Table (4) Effect of reaction time (min) on the formation of reducing sugars.

| Hydrolytic treatment | | | | | Lignocellulosic materials | | | | | |
|----------------------|---------------------|--------------------|-------------------|-------------------|---------------------------|--------|---------------------------|--------|------------------------|--------|
| Acid Conc. (%) | Solid: liquid (W/V) | React. time (min.) | Soak. time (hour) | React. temp. (°C) | Rice straw ^b | | Cotton stalk ^c | | Beet pulp ^a | |
| | | | | | RS (g/L) | SR (%) | RS (g/L) | SR (%) | RS (g/L) | SR (%) |
| 4 | 1 : 20 | 20 ^E | 12 | 121 | 52.08 | 61.4 | 33.56 | 64.4 | 54.56 | 62.4 |
| | | 30 ^D | | | 53.60 | 66.1 | 34.34 | 67.4 | 55.10 | 67.0 |
| | | 60 ^C | | | 55.70 | 66.0 | 34.80 | 67.0 | 55.83 | 55.5 |
| | | 90 ^A | | | 56.63 | 64.5 | 34.76 | 65.4 | 58.53 | 59.0 |
| | | 120 ^B | | | 56.55 | 62.8 | 34.70 | 63.7 | 58.50 | 62.7 |

*Each value was average of three determinations.

RS = reducing sugar (g/ 100 g sample)

SR = solid recovered (g/ 100 g sample)

a, b and c: comparison of means of reducing sugar % by type of waste.

A, B, C and D: comparison of means of yield % by reaction time (min.).

Means within a column not sharing superscript are significantly different (P <0.01).

Effect of soaking time (hour) on the formation of reducing sugars.

The effect of acid pretreatment time on the formation of reducing sugars from rice straw, cotton stalk and beet pulp are given in Table (5). The data show that, with increasing of the soaking time from 12 hours to 36 hours, reducing sugars in the three wastes were increased with the increasing of the time. The increasing of reducing sugar content may be attributed to the increasing of the pretreatment time where the solubility of lignin, hemicellulose and silica was increasing. From the same table data show that the most effective time was 36 hours which give reducing sugar content of 58.33, 35.65 and 58.85% for rice straw, cotton stalk and beet pulp, respectively. These results are in agreement with Sun *et al.* (2002).

Table (5) Effect of soaking time (hour) on the formation of reducing sugars.

| Hydrolytic treatment | | | | | Lignocellulosic materials | | | | | |
|----------------------|---------------------|--------------------|-------------------|-------------------|---------------------------|--------|---------------------------|--------|------------------------|--------|
| Acid conc. (%) | Solid: liquid (W/V) | React. time (min.) | Soak. time (hour) | React. temp. (°C) | Rice straw ^b | | Cotton stalk ^c | | Beet pulp ^a | |
| | | | | | RS (g/L) | SR (%) | RS (g/L) | SR (%) | RS (g/L) | SR (%) |
| 4 | 1 : 20 | 90 | 12 ^D | 121 | 56.63 | 64.5 | 34.76 | 65.4 | 58.53 | 59.0 |
| | | | 24 ^C | | 56.74 | 62.5 | 35.47 | 60.4 | 58.57 | 61.8 |
| | | | 36 ^A | | 58.33 | 61.4 | 35.65 | 59.5 | 58.85 | 68.0 |
| | | | 48 ^B | | 58.23 | 54.3 | 35.79 | 58.1 | 58.73 | 64.7 |

*Each value was average of three determinations.

RS = reducing sugar

SR = solid recovered (g/ 100 g sample)

a, b and c: comparison of means of reducing sugar % by type of waste.

A, B, C and D: comparison of means of yield % by soaking time (hour).

Means within a column not sharing superscript are significantly different (P <0.01).

CONCLUSIONS

The optimal reaction conditions, which resulted in an acid hydrolysis, were (1:20) (w/v) solid to liquid ratio; 121°C hydrolysis temperature; 4% (w/v) acid concentration and 90min reaction time and pre-soaked for 36 hours. The produced hydrolysate can be useful for fermentation processing for example to produce ethanol.

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انتاج الايثانول من بعض مخلفات التصنيع الزراعي : ١. المعاملات الأولية التي تجرى على بعض المواد الخام لاستخدامها في عمليات التخمر.
أمين كمال عمار و سمير يوسف السناط
قسم تكنولوجيا الأغذية – كلية الزراعة – جامعة كفر الشيخ – مصر

تهدف هذه الدراسة إلى الاستفادة من المخلفات الزراعية والصناعية باعتبارها مواد وسطية لاستخدامها في الصناعات التخمرية ، في نفس الوقت التخلص من الأثر البيئي السيئ الناتج من حرق هذه المخلفات في الحقول. تم في هذه الدراسة تعريض كل من مخلفات قش الأرز، وحطب القطن ولب البنجر المتخلف من صناعة سكر البنجر، لمحلول حمضي مخفف في درجة حرارة عالية لتمكين إنتاج أقصى قدر ممكن من السكريات القابلة للتخمر من التحلل الحمضي. وتمت دراسة تأثير مختلف المتغيرات بما في ذلك درجة حرارة المعالجة، ووقت التفاعل، تركيز الحامض، نسبة المواد الصلبة إلى السائل ومدة النقع على تحرير السكر. وقد أجري التحليل المائي الحامضي باستخدام تركيزات من حمض الكبريتيك تراوحت من ١-٥% (وزن/حجم) وتراوحت نسبة الصلب إلى السائل من ١:٥ حتي ١:٢٥ (وزن/حجم) في ١٢١ م وتراوحت زمن التفاعل من ٣٠-١٢٠ دقيقة. وأوضحت النتائج أن هناك فروق جوهرية في التركيب الكيميائي للمواد الثانوية موضوع الدراسة. كما أوضحت الدراسة أن الظروف المثلى لتحرير السكريات القابلة للتخمر تحت الظروف المختبرة وعلى ١٢١ م، كانت تركيز حامض ٤% ونسبة صلب: سائل (٢٠:١) ومدة تفاعل قدرها ٩٠ دقيقة والنقع لمدة ٣٦ ساعة.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
كلية الزراعة – جامعة كفر الشيخ

أ.د / احمد عبد العزيز الرفاعي
أ.د / سمير محمود متولي

