PRODUCTION OF GLUCOSE AND ETHANOL FROM SOME CELLULOSIC RESIDUES USING THE PRODUCED CELLULASE ENZYME FROM T. reesei, T. harzianum AND A. niger.

BY


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ABSTRACT

The lignocellulosic materials, faba bean straw and green pea pods were chemically pretreated by sodium hydroxide or glacial acetic acid. The amount of cellulose residues from faba bean straw and green pea pods decreased gradually by increasing NaOH concentration under conditions of 3 h, 100°C and 48h, 25°C. While, pretreatment of faba bean straw with glacial acetic acid showed higher yield of cellulose (80% and 70%) under the same above conditions. The chemical composition of faba straw and green pea pods were determined. Cellulose, hemicellulose and lignin contents were 32.3, 23.0 and 13.1% for crude faba bean straw and 29.2, 20.4 and 10.6% for crude green pea pods. After pretreatment, the extracted cellulose, hemicellulose and lignin contents were found to be (72.5, 10.5 and 7.2%) in faba bean straw and (80.0, 8.1 and 4.8%) in pea pods, respectively. The rate of saccharification process were 62.2% by T. reesei, 59.9% by T. harzianum and 56.2% by A. niger, for extracted cellulose of faba bean straw at concentration of 8% and the period of reaction was 6h. While, the saccharification rates were to 66.3, 58.8 and 65.0% by using the same above-mentioned fungi for extracted cellulose of green pea pods residue at concentration of 10% after 6 h.

Production of ethanol by fermentation of the resultant glucose which produced from saccharification processes amounted to 89.5, 84.7 and 85.7% by using hydrolyzed carboxymethyl cellulose of T. reesei, T. harzianum and A. niger, respectively. While, the ethanol produced from saccharification processes of faba bean straw and green pea pods estimated to (89.5, 89.5 and 88.5%) and (83.6, 89.5 and 88.0%) respectively by using produced cellulase from the above-mentioned fungi.

INTRODUCTION

Lignocellulosic waste materials are considered as a most important renewable reservoir of carbon. Wood and forestry waste and agricultural residues are abundant feedstocks for saccharification and subsequent
conversion to food, fuels and chemicals. (Deschainps, et al., (1985). The application of cellulytic enzymes for the bioconversion of lignocellulosic residues into fermentable sugars can be used as chemical feedstocks for the production of industrial chemicals including fuel alcohols.

Hoda, et al., (1990) used the enzyme culture filtrate of \textit{P. funiculosum} for saccharification of the cellulosic wastes (pea hulls, rice straw, carrot leaves and guava seeds). The maximum amount of saccharification process was 30.29, 39.99, 15.87 and 8.44 g/L, respectively after 65 h with 5% substrate concentration.

Gabr, et al., (1991, b) treated wheat straw and cotton stalks with peracetic acid. They noticed that peracetic acid is highly effective on rice straw. Also, they observed that the rate of saccharification of all cellulosic materials under their studies was directly proportional to the concentration of peracetic acid.

Abd El-Malak (1995) studied the enzymatic hydrolysis of some agricultural by-products, pretreated by NaOH and NH$_4$OH (1.0M, 24h at room temperature). The highest value of saccharification process was 89.8% from the extracted cellulose of soybean straw at concentration of (2.0%) after 48h.

Okeke and Obi (1995) studied the enzymatic hydrolysis of some agrowastes under the effect of two fungi isolates i.e. \textit{Sporotrichum pruinosum} and \textit{Arthrographis sp.} They mentioned that the highest degrees of hydrolysis 15.1% for \textit{Sporotrichum pruinosum} and 7.5% for \textit{Arthrographis sp.} were observed with alkali pretreated seed shells as substrate.

Szczodrak (1988) used the pretreated wheat straw for production of ethanol at 43 °C in the simultaneous saccharification and fermentation (SSF) process by \textit{T. reesei} cellulase and kyveromyces fragilis. They obtained 2.4% (w/v) ethanol from 10% (w/v) of chemically treated straw after 48 h.

Gunasekaran and Kamini (1991) found that ethanol production by \textit{Kluyveromyces fragilis} was 65 g/L using 150 g/L lactose while, \textit{Zymminas mobiles} produced 72 g ethanol/L under the same conditions.

Ahuja et al., (1992) used the enzymatically hydrolyzate of rice straw to produce ethanol with \textit{Saccharomyces cerevisia}. The maximum yield of ethanol was (4.12 % v/v) after 36 h incubation.

Lisbethsson and Bärbel, (1996) found that ethanol produced from lignocellulosic hydrolyzates in an economically feasible process requires micro-organisms that produce ethanol with a high yield from all sugars present, and have a high ethanol productivity from lignocellulosic hydrolysate.

Moritz and Duff (1996) found that ethanol productivity was increased up to 65% over conventional (nonextra-reactive) fed-bach simultaneous saccharification of cellulosic substrates. In batch simultaneous saccharification
and extractive fermentation (SSEF) reactors with 2.5% aqueous phase, 50% conversion of 25% (aqueous phase concentration) Solka Floc could be achieved in 48 h using 2 FPU/g cellulose.

The aim of the present work is to study the conversion of some locally-available agro-waste materials (faba bean straw and green pea pods) by using different fungi cellulases (T. reesei, T. harzianum and A. niger) to soluble sugars and ethanol.

**MATERIALS AND METHODS**

1. **Sources of cellulosic substances:**
   Faba bean straw (*Vicia faba*) and pea pods (*pisum sativum*) were utilized in this study. Faba bean straw was obtained from the experimental station at Nobaria, Agricultural Research Centre. However, green pea pods were obtained from Montana Co., for Food Industry, Qalama, Qaliub, Qaliubia Governorate, Egypt.

2. **Chemical analysis of Faba bean straw and green pea pods:**
   Moisture content, ash, total lipids total nitrogen and crude protein Values were determined according to the methods described by A.O.A.C (1980). Lignin content was determined gravimetrically by the method reported by Adams (1965). Cellulose and hemicellulose contents were achieved according to the method described by Chen and Anderson (1980). Total carbohydrates were estimated by the method reported by Montgomery (1961). Total reducing sugars were determined according to Somogyi (1952) by using the chromogenic reagent of Nelson (1944).

3. **Pretreatment of lignocellulosic materials:**
   The milled agricultural wastes (Faba bean straw and green pea pods) were subjected to various chemical concentrations of NaOH and glacial acetic acid. The concentrations of NaOH solutions were 1, 2, 3, 4, and 5% (w/w) while, the concentrations of glacial acetic acid solutions were 4, 8, 12, 16, 18% (v/w).

   The pretreatment of lignocellulosic materials were accomplished according to the method described by Chen and Anderson (1980) under different conditions 3 h at 100°C and 48 h at room temperature.

4. **Enzyme assays:**
   Enzyme assays were carried out on culture filtrates at pH 4.8. (0.1 M citrate buffer) and 50°C. The activity was expressed as micromoles of reducing sugars. Carboxymethyl cellulase (CMC-ase) and Filter paper-ase (FP-ase) activities were measured according to the method described by Tanaka, et al., (1985).
5. Saccharification process:

The saccharification process was carried out by the method described by Szczodrak (1988). The percentage of saccharification (%) was calculated using the following equation as reported by Okeke and Obi (1995).

\[
\text{Saccharification} \% = \frac{\text{amount of reducing sugar (mg)}}{\text{amount of substrate (mg)}} \times 0.9 \times 100
\]

6. Alcohol production from the lignocellulosic materials

The method of Ban and Han (1990) was used to produce the alcohols from CMC, Faba bean straw and green pea pods. The resultant alcohol was determined by gas chromatography (GC) HP 5890 F. Spectrophotometer with refractive index detector and amines HP5 column (cross linked 5% pH Mesilion 30m × 0.32mm × 0.25mm) Flm.

RESULTS AND DISCUSSION

1. Chemical Pretreatment of lignocellulosic Materials:

Pretreatment of lignocellulose can enhance its hydrolysis by increasing the accessibility of its components cellulolytic enzyme.

Sodium hydroxide (NaOH) and glacial acetic acid were used to increase the susceptibility of lignocellulosic crop residues. The pretreatment with (NaOH) remove a large amounts of hemicellulose and lignin, which increase the accessibility to the cellulase, and improve the fiber to be more permeable to cellulase.

The effect of sodium hydroxide (1.0-6.0 w/w) and glacial acetic acid (4.0-20.0 v/v) on the extraction of cellulose and hemicellulose in Faba bean straw and green pea pods at different time and temperature are tabulated in Tables (1) and (2).

The obtained results showed that the amount of cellulose residues from Faba bean straw and Pea pods decreased gradually by increasing (NaOH) concentration under conditions of 3h, 100°C and 48 h. 25°C.

Also, pretreatment of Faba bean straw with glacial acetic acid in Table (2) showed higher yield of cellulose (80 %, 70 %) at 3h, 100°C and 48h, 25°C respectively with 4 % glacial acetic acid, compared with green pea pods which yielded 63.3 % and 68.0 % under the above-mentioned conditions. The obtained results are in agreement with those obtained by Gabr, et al., (1991, b).

The results in Table (1) showed that the highest obtained value i.e. (80 %) of extracted cellulose from pea pods was higher than that of Faba bean straw which contained 72.5 % at the same conditions (3h, 100°C). On the other hand, the amount of isolated hemicellulose from pea pods reached its highest content (28.1 %) at 5 % NaOH while, Faba bean straw contained less amount (27.5%) under the same conditions. This observation may be due to
the loss of significant amount of hemicellulose in the washing stage under high NaOH concentration, (Fox, et al., 1989).

Table (1): Effect of NaOH concentration, temperature and time on the extraction of cellulose and hemicellulose from lignocellulosic materials.

<table>
<thead>
<tr>
<th>NaOH Conc. (w/w %)</th>
<th>Faba bean straw</th>
<th>Green pea pods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose %</td>
<td>Hemicellulose %</td>
</tr>
<tr>
<td></td>
<td>3h, 100°C</td>
<td>48h, 25°C</td>
</tr>
<tr>
<td>1</td>
<td>72.5</td>
<td>65.0</td>
</tr>
<tr>
<td>2</td>
<td>70.0</td>
<td>60.0</td>
</tr>
<tr>
<td>3</td>
<td>67.5</td>
<td>57.5</td>
</tr>
<tr>
<td>4</td>
<td>65.0</td>
<td>55.0</td>
</tr>
<tr>
<td>5</td>
<td>60.0</td>
<td>50.0</td>
</tr>
<tr>
<td>6</td>
<td>54.4</td>
<td>44.6</td>
</tr>
</tbody>
</table>

Table (2): Effect of glacial acetic acid, temperature and time on the extraction of cellulose and hemicellulose from lignocellulosic materials.

<table>
<thead>
<tr>
<th>Glacial acetic acid (%) (w/w %)</th>
<th>Faba bean straw</th>
<th>Green pea pods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose %</td>
<td>Hemicellulose %</td>
</tr>
<tr>
<td></td>
<td>3h, 100°C</td>
<td>48h, 25°C</td>
</tr>
<tr>
<td>4</td>
<td>80.0</td>
<td>70.0</td>
</tr>
<tr>
<td>8</td>
<td>73.3</td>
<td>60.0</td>
</tr>
<tr>
<td>12</td>
<td>66.7</td>
<td>50.0</td>
</tr>
<tr>
<td>16</td>
<td>60.0</td>
<td>43.0</td>
</tr>
<tr>
<td>18</td>
<td>50.0</td>
<td>38.0</td>
</tr>
<tr>
<td>20</td>
<td>46.8</td>
<td>30.0</td>
</tr>
</tbody>
</table>

On the other hand, cellulose content in this study was decreased by increasing the NaOH concentration. The acquired results are in a good harmony with those found by Diwamy et al., (1986). The obtained results may be due to that NaOH led to breakdown of intermolecular hydrogen bond of lignocellulosic fibers which promote the swelling of fibers beyond water-swollen dimension and thereby increasing enzymatic and microbiological penetration into the cell wall fine structure (Tarkaw and Feist, 1969). Also, alkali treatment disrupts or breaks the bonds of lignin moieties and reduces lignin content (Blanch and Wilke, 1982).

2. Chemical composition of lignocellulosic materials:-

The chemical composition of lignocellulosic materials i.e. Faba bean straw and green pea pods are tabulated in Table (3). The obtained results
showed that cellulose, hemicellulose and lignin are the main constituents while ash and protein occur in lesser amounts in crude and extracted lignocellulosic materials under investigation. The results in Table (3) show that crude Faba bean straw contained the highest amount of cellulose (32.3%) while, crude pea pods contained lesser amount (29.2%). The lignin amounted to 13.1% in Faba bean and 10.6% in pea pods.

On the other hand, pretreatment of lignocellulosic materials with NaOH under the above-mentioned conditions increased the amount of isolated cellulose and decreased the amount of isolated hemicellulose and lignin content. The extracted cellulose, hemicellulose and lignin contents after this pretreatment were found to be (72.5%, 10.5% and 7.3%) in Faba bean straw. Also, the extracted cellulose, hemicellulose and lignin were found to be (80.0%, 8.1% and 4.8%) in pea pods.

Table (3): Chemical composition of crude and extracted faba bean straw and pea pods.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Faba bean straw</th>
<th>Pea pods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Extracted</td>
</tr>
<tr>
<td>Moisture</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Cellulose</td>
<td>32.3</td>
<td>72.5</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>23.0</td>
<td>10.5</td>
</tr>
<tr>
<td>Lignin</td>
<td>13.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Ash</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Protein</td>
<td>3.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Total Lipids</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>Total hydrolysable carbohydrate</td>
<td>13.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Total soluble sugar</td>
<td>2.6</td>
<td>-</td>
</tr>
</tbody>
</table>

Percentage on dry weight basis under experimental conditions

From the obtained results, it could be concluded that the pretreatment of lignocellulosic materials with NaOH decreased the lignin to a large extent. Consequently, cellulase enzyme became more effective towards the extracted Faba bean straw and pea pods, Fox et al., (1989).

3. Effect of different treatments of cellulosic materials on enzymatic saccharification:-

Saccharification processes were applied on different extracted cellulose from lignocellulosic material i.e. Faba bean straw and green pea pods at substrate concentration of (2.0, 4.0, 6.0, 8.0 and 10.0% w/v) using (0.1M) acetate buffer solution pH 5.0. These processes were carried out by utilizing cellulase enzyme produced by T. reesei, T. harzianum and A. niger at enzyme concentration of 54.5, 40.6, 51.3 units protein enzyme. The experiments were achieved in a shaking incubator at 200 r.p.m and 50°C.

The rate of enzymatic saccharification was accomplished for different periods ranged between 3h and 6h. Saccharification of different
treated lignocellulosic materials are recorded in Tables (4, 5) and Figs (1, 2). The results in (Table 4) indicated that, the highest value of saccharification process were 46.9% by T. reesei, 43.7% by T. harzianum and 46.4% by A. niger, for the extracted cellulose of Faba bean straw at concentration of 8% and the period of reaction 3 hrs. On the other hand, at the same concentration of extracted cellulose from Faba bean straw, the saccharification percentage amounted to (62.2, 56.4 and 56.0 and 56.0% %) by using T. reesei, T. harzianum and A. niger, respectively for a period of 6hrs. These results are in agreement with those previously obtained by Vega et al., (1991) and Okeke and Obi, (1995).

### Table (4): Enzymatic saccharification of treated Faba bean straw.

<table>
<thead>
<tr>
<th>Source of Enzyme</th>
<th>3 hrs Substrate concentration (% w/v)</th>
<th>6 hrs Substrate concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>T. reesei</td>
<td>36.7</td>
<td>38.6</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>25.8</td>
<td>34.3</td>
</tr>
<tr>
<td>A. niger</td>
<td>23.4</td>
<td>35.0</td>
</tr>
</tbody>
</table>

### Table (5): Enzymatic saccharification of treated pea pods.

<table>
<thead>
<tr>
<th>Source of Enzyme</th>
<th>3 hrs Substrate concentration (% w/v)</th>
<th>6 hrs Substrate concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>T. reesei</td>
<td>38.2</td>
<td>54.0</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>30.8</td>
<td>43.0</td>
</tr>
<tr>
<td>A. niger</td>
<td>27.6</td>
<td>35.9</td>
</tr>
</tbody>
</table>

However, the maximal improvement in the saccharification rate of any cellulosic material under this investigation is still relatively low. This observation may be attributed to that the surface area of cellulose is not major limiting factor but the crystallinity of cellulosic materials might play a bigger limiting rate as illustrated by Fan, et al., (1980). In addition the poor improvement of physical and chemical pretreatments might be due to its incapability to remove the lignin which accounts for the most hinderance towards saccharification process. Also, the end-product inhibition and to
Fig (1): Enzymatic saccharification of treated faba bean straw

Fig (2): Enzymatic saccharification of treated pea pods.
some extent thermal instability during the long reaction period. Okeke and Obi (1995). Also, from the obtained data which are shown in table (5) and fig (2), it might be concluded that the maximum values of saccharification process were 62.7, 55.8 and 56.0% by using T. reesei, T. harzianum and A. niger respectively, at concentration 10% and period of 3h. While at the same concentration of the extracted cellulose from pea Pods, the saccharification percentage equalled to 66.3, 58.8 and 65.0% respectively, by the same fungi after 6 h. The obtained data is coincident with that reported by Vega et al., (1991).

However, the results pointed that, the extracted cellulotic materials from green pea pods achieved maximum saccharification values higher than Faba bean straw. This observation may be attributed to that the extracted cellulose from pea pods contained a less amount of hemicellulosic polysaccharides which act as impurities in the saccharification process. And hence, these polymers led to decrease the saccharification values of treated Faba bean straw.

4. Production of ethanol by fermentation of lignocellulosic materials:-
Fermentation of the lignocellulosic hydrolyzate to ethanol was accomplished as an ultimate step in the present biocconversion scheme. The starting materials used here were the hydrolyzates attained from the enzymatic degradation under the optimal saccharification condition. The unusual yeast strain Saccharomyces cerevisia (V-21) was chosen to ferment these hydrolyzed products, since it is capable of producing ethanol from CMC, treated Faba bean straw and treated pea pods, Delgenes, et al., (1996). To allow a clear evaluation of the ethanol productivity of this yeast, it was grown on variable carbon sources including CMC, Faba bean straw and pea pods by 5.0 g/100 glucose. The results are shown in Table (6) indicated that the high efficiency of the present yeast for conversion of CMC and treated faba bean straw into ethanol by T. reesei, T. harzianum A. niger were 89.5%, 84.7%, and 85.7% respectively. These results are in agreement with those previously reported by Roca, et al., (1996).

<table>
<thead>
<tr>
<th>Source of straw</th>
<th>Ethanol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T. reesei</td>
</tr>
<tr>
<td>CMC</td>
<td>89.5</td>
</tr>
<tr>
<td>Faba bean</td>
<td>89.5</td>
</tr>
<tr>
<td>Pea pods</td>
<td>83.6</td>
</tr>
</tbody>
</table>

Table (6): Fermentation of the lignocellulosic hydrolyzates to ethanol.
REFERENCES


إنتاج الجلوكوز والإيثانول من بعض المخلفات السليفووزية باستخدام إنزيم السليفووزي المنتج من تراي ريزيا، تراي هيرننيم والأسرجلاس نيجر

صلاح مصطفى محمود سعد، فرحات فودة، فودة

عزت رزق جندي، نظمي زكي

قسم الكيمياء الحيوية الزراعية - كلية الزراعة - جامعة الزقازيق - قسم البيولوجيا - قسم الميكروبيولوجي

مركز البحوث الزراعية - قسم الميكروبيولوجي

بهدف هذا البحث، دراسة المواد اللجنوسيلوفوزية الخاصة بمخلفات تشغيل الفول البلدي، وبقايا قرونة البسيلة لإنتاجها في إنتاج مواد لها قيمة غذائية مثل شراب الجلوكوز، ومنتج الأليافكدة والمصلوب للطاقة. وقد استخدمت الصودا الكاوية، وحامض الخليك النيتي، لإعداد الخليق السليفووزي. وتفكرت البوراجي هذة الجزيئات لتحويلها إلى صورة غير بذرية حتى يسهل إنزيم السليفووزي مهاجماتها وتحليلها.

وقد أظهرت النتائج أن معاملة هذة المخلفات بحامض الخليك النيتي (4%) حجمي (وزني) أدى إلى النتائج 80% من السليفووز المستخلص بنسبة 75% لكلغة البسيلة، وذلك على درجة الحرارة 100 درجة مئوية لمدة 25 دقيقة في 48 ساعة. في حين، بينما بقايا قرون البسيلة كفاك السليفووز المستخلص 80% عند معاملتها بالصودا الكاوية (1% وزني/وزنين) على نفس الظروف السابقة.

وقد تم دراسة المكونات الأساسية لهذه المخلفات السليفووزية وأظهرت النتائج المتاحفة، أن قبل الدواء السليفووزي الخام تحتوي على 32% من السليفووز، 23% هيدرسليفووز، 13% لجيني، بينما نسبة هذه المكونات في بقايا قرون البسيلة الخام كانت 29%، 40% و10% على التوالي. وبعد المعالجة الكيميائية لمخلفات تشغيل الفول البلدي، وبقايا قرون البسيلة بواسطة الصودا الكاوية، تحت الظروف السابقة، كانت نسبة السليفووز، هيدرسليفووز، المستخلص من الفول البلدي 57%، 86%، 67%، 10%، 8%، 4% في حالة قرون البسيلة المستخلص من الفول البلدي، وبقايا قرون البسيلة لمدة 3، 6، 9، 12 ساعة بتركيز 2، 4، 6، 8، 10% من المواد المتفاعلة.

وقد تم إجراء عملية التسكيك لإنتاج السكريات تحت الظروف المثلى للفول البلدي، وبقايا قرون البسيلة لمدة 3، 6، 9، 12 ساعة بتركيز 2، 4، 6، 8، 10% من المواد المتفاعلة.
وأظهرت النتائج المتحصل عليها أن أعلى معدل للتمشيط فـى 6 ساعات 
وذلك في وجود الإندماج المنتج من فطور 
(ق2، 2 ل6، 2 ل6، 2 ل6، 2 ل6) بتركيز 8% من السليولوز المستخلص من قشر الفول 
البلدى بينما كان أعلى معدل للتمشيط للسليولوز المستخلص من قرون البذور 2 ى، 2 8 
65% وذلك باستخدام نفس الإندماجات المنتجة من الفطرية السابقة في 6 
ساعات بتركيز 10% من المادة المتفاعلة تحت نفس الظروف المثلد للتحلـيل 
الإندميم.

وقد تم دراسة إنتاج كحول الإثيلى من تخمر السكريات الناتجة من عملية 
التمشيط بواسطة خميرة سكراخموميس سيرفيش 
وقد أوضحت النتائج أن نسبة التحويل كانت 69، 4% 62، 1% من الجلوكوز الناتج من الكربي كم ميشيل 
A. niger ' T. harzianum 'T. reesei سـيولوز للانزيمات الناتجة من فطور 
على التوالي بينما كانت نسبة التحويل من الجلوكوز الناتج من قشر الفول البلدى 
(69، 4% 62، 1%) وباقيا قرون البذور (62، 1% 68، 2%) بـاستخدام 
نفس الإندماجات الناتجة من الفطرية السابقة الذكر.