

## Treatment of Waste Paper Using Ultrasound and Sodium Hydroxide for Bioethanol Production

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### Abstract

Bioethanol produced from lignocellulose feedstock is a renewable substitute to declining fossil fuels. Pretreatment using ultrasound assisted alkaline was investigated to enhance the enzyme digestibility of waste paper. The pretreatment was conducted over a wide range of conditions including waste paper concentrations of 1-5%, reaction time of 10-30 min and temperatures of 30-70°C. The optimum conditions were 4 % substrate loading with 25 min treatment time at 60°C where maximum reducing sugar obtained was 1.89 g/L. Hydrolysis process was conducted with a crude cellulolytic enzymes produced by *Cellulomonas uda* (PTCC 1259). The maximum amount of sugar released and hydrolysis efficiency were 20.92 g/L and 78.4 % , respectively. Sugars released from waste paper were fermented into bioethanol with *Saccharomyces cerevisiae*. The maximum concentration of bioethanol estimated was 9.5 g/L after 48h of cultivation, the yield and volumetric productivity were 0.454 g/g glucose and 0.2g bioethanol/ L h. respectively. This study of ultrasound and sodium hydroxide treatment may be (we think) it will be a promising technique to develop bioethanol production from waste paper.

**Keywords:** Bioethanol, *Cellulomonas uda*, ultrasound, waste paper

### المخلص

الايثانول الحيوي المنتج من المخلفات اللكنوسليلوزية يعتبر بديل طاقة متجدد لاستبدال الوقود الاحفوري. تم دراسة المعاملة باستخدام الامواج فوق الصوتية وبمساعدة القاعدة لتحسين عملية التحلل الانزيمي للمخلفات الورقية. تم استخدام ظروف مختلفة اثناء الدراسة لعدة متغيرات شملت (تركيز المخلفات الورقية. وقت المعاملة ودرجة الحرارة). الظروف المثالية التي تم الحصول عليها كانت 4 % تركيز المخلفات ومعاملة السكريات المختزلة عند هذه الظروف *Cellulomonas uda* (PTCC 1259) لمدة 25 دقيقة عند درجة حرارة 60 درجة مئوية وكان تركيز 1.89 غرام لكل لتر. تم استخدام بكتيريا لانتاج الانزيم الخام لغرض اجراء عملية التحلل الانزيمي وكان اعلى تركيز للسكريات المختزلة الناتجة السكريات المنتجة من المخلفات الورقية تم تخميرها بواسطة *S. cerevisiae* 20.92S. غرام لكل لتر. لانتاج الايثانول الحيوي. اعلى تركيز خميرة للايثانول تم الحصول عليه بعد 48 ساعة من التخمير هو 9.5 غرام لكل لتر. دراسة المعاملة بالامواج فوق الصوتية كانت عملية مؤثرة وناجحة في انتاج الايثانول الحيوي.

الكلمات الدالة: الايثانول الحيوي، الامواج فوق الصوتية، المخلفات الورقية

### Introduction

Increasing energy costs together with increasing concerns about global warming related to CO<sub>2</sub> emissions and the decrease of landfill locations for disposal of solid waste resulted in increasing interest in alternative, low and non-carbon based energy sources [1-4]. Lignocellulosic are the most abundant biomass available on earth, comprising mainly of cellulose and hemicellulose [5,6]. Among various lignocellulosic, waste paper could be used as an excellent source for bioethanol production; it is abundant, low cost with high amount of cellulose and does not require energy-intense thermophysical or severe strong acid pretreatments prior to enzymatic hydrolysis generally used in hydrolysis of lignocellulose substrates [3,7]. Bioethanol derived from biomass is the only liquid transportation fuel that does not contribute to the greenhouse gas effect, it yields zero net CO<sub>2</sub> output into the atmosphere because after burning of bioethanol, the released CO<sub>2</sub> is recycled back into plant material because plants use CO<sub>2</sub> to synthesize cellulose during photosynthesis. There for no net CO<sub>2</sub> is added to the atmosphere, making bioethanol an environmentally valuable energy source [2,8,9]. Previous researches have demonstrated the potential of bioethanol production from waste paper using a variety of process designs [6,10-14]. Currently, the technology for lignocellulosic bioethanol production relies mainly on pretreatment, hydrolysis and fermentation [15]. Because of structural complexity, pretreatment is required to disrupt the intractable structure of lignocellulosic materials and to increase the accessibility of hydrolytic enzymes to the carbohydrates polymers. There are several types of pretreatment methods including acid, alkali, organic solvent,

hydrogen peroxide, ammonia and liquid hot water treatment [11]. The choice of pretreatment process is possibly the most important factor in the economics of bioethanol production process because it affects waste treatment, cellulose conversion rates and mainly hydrolytic enzymes performance [16]. The application of ultrasonic technology in lignocellulosic biomass pretreatment has concerned the attention of the educational community. Ultrasonic helps in enhancing the mass transfer rates due to the generation of turbulence and sound streaming which also enhance delignification process by increased penetration of solvent into substrate [17]. In this sense, ultrasonic can be successfully used to develop the pretreatment process by reducing the structural rigidity of lignocellulosic biomass and by reducing the mass-transfer resistances, which can lead to enhanced hydrolysis step, increased product yield with reduced processing time and enzyme consumption, based on these features, the main objective of this study was to determine the optimum conditions for waste paper treatment using ultrasound and sodium hydroxide.

### Materials and Methods

Waste paper, which was used as a substrate for the production of bioethanol, was collected from the Biochemical Engineering Department/ Baghdad University. The collected waste papers were taken for size reduction using a standard shredder (SUNWOOD, China). Its composition on an oven dry basis was 60 % glucan, 15.2 % hemicellulose, 4.3 % moisture, 9.7 % ash and 5.4 % lignin. The composition was determined by using standard method described by NREL [18-20]. For ultra-sonic pretreatment a laboratory scale ultrasonic (Elmasonic, Germany) was used. 3,5-Dinitrosalicylic acid (DNSA), sodium hydroxide and other chemicals were obtained as a gift sample from Biotechnology Lab. / Alkharizmi college of engineering/ University of Baghdad. Bacteria *Cellulomonas uda* (PTCC 1259) was obtained from the Persian Type Culture Collection (PTCC).

#### Experimental methods

##### Pretreatment of waste paper

The first stage of pretreatment was done by presoaking of waste paper in distilled water at room temperature in the ratio of 1:20 and it stands for 24h. During this period, the fibers of the paper are loosening and it makes easier to separate the cellulose, hemicelluloses and lignin components from the fiber of the papers [21]. Then water was filtered and the waste paper was dried overnight in an oven at 50°C, after that waste paper was grounded in electric blender to form fluffy wool, this dried substrate was used as a raw material for the next steps.

Delignification was the second stage of pretreatment, it includes ultrasound assisted alkaline treatment for delignification of the waste paper, substrate was treated with 0.1N NaOH and ultrasonicated using a laboratory scale ultrasonic (37 kHz, 280 W) as shown in Figure (2). Conditions for the (US) pretreatment of waste paper were optimized by varying substrate loading 1, 2, 3, 4 and 5 % w/v, pretreatment time 10, 15, 20, 25 and 30 min and pretreatment temperature 30, 40, 50, 60 and 70 °C.

##### Production of Cellulolytic Enzyme

The spore suspensions of *Cellulomonas uda* (PTCC 1259) was used as inoculum for production of cellulase, the medium used for production of enzyme, the medium used for production of enzyme contains in g/L: CMC (10), Peptone (10), K<sub>2</sub>HPO<sub>4</sub> (2), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.25), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (2.5) [22], Production medium was sterilized by autoclaving at 121°C for 20 min with pH adjusted to (7), after cooling down at room temperature, the medium was injected with 10 % (v/v) of vegetative cells of bacterial isolates and incubated in a shaking incubator at 30°C for 48 h with 150 rpm shaking speed. After 48 h of incubation, the broth was centrifuged at 10000 (rpm) at 4 °C for 10 min, the clear supernatant gained was used as crude enzyme.

##### Hydrolysis of the pretreated waste paper

The produced cellulose after pretreatment process was converted into reducing sugar utilizing the cellulose degrading bacteria. Enzymatic hydrolysis was done using 5 % (v/v) of crude enzyme previously prepared at 40°C with pH adjusted to 5 in an orbital shaker 150 rpm for 48 h. Samples were taken every 12h for reducing sugar analysis.

##### Fermentation of waste paper hydrolysate

A filter-sterilized hydrolysate obtained from enzymatic hydrolysis of waste paper was used as a substrate for production of bioethanol with no glucose supplementation using separate hydrolysis and fermentation (SHF). Fermentation was performed in 500 mL flasks containing 300 mL of fermentation medium contains in (g/L): Yeast extract (3) and Peptone (5), production medium was sterilized by autoclaving at 121°C for 20 min with pH adjusted to (5) and allowed to cool down at room temperature. Flasks were inoculated with 10% (v/v) of

*S. cerevisiae* inoculum obtained from growth for 24h on medium contained in (g/L): Glucose (10), Peptone (5), Yeast extract (10),  $K_2HPO_4$  (3) [23]. During the reaction, samples were withdrawn every 12h from zero point for the analysis of bioethanol produced and sugar consumed.

#### Analysis

The reducing sugars (TRS) was analyzed using dinitrosalicylic acid (DNS) method [24]. The absorbance was measured at 540 nm with UV- Vis spectrophotometer (SHIMADZU, Japan). Bioethanol produced was analyzed by High Performance Liquid Chromatography (HPLC) (Knauer, Germany) equipped with a reflective index detector Smart line a Eurokat H, Knauer column. Samples were run at 75°C and eluted at 0.5 ml/min with 0.01N  $H_2SO_4$ . The tests were conducted in duplicate.

#### Results and discussion

##### Effect of substrate loading

Pretreatment of different substrate loading 1 to 5 % w/v was carried out with 0.1 N NaOH for 20 min at 50°C. The graphical representation of the results can be seen in Figure (1). The maximum (TRS) obtained was 1.43g/l at 4% waste paper concentration. An initial increasing of (TRS) formation was noticed when waste paper percentage increased from 1 to 4%, at 5% concentration the reducing sugar decreased because of mass transfer obstacles due to increasing viscosity of medium.

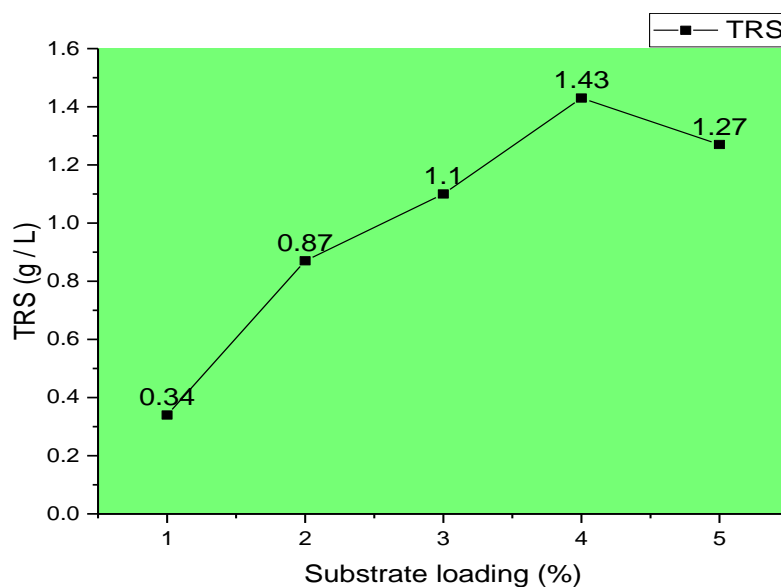


Fig. (1): Effect of substrate loading on TRS

##### Effect of pretreatment time

Pretreatment of waste paper was carried out with different pretreatment time 10, 15, 20, 25 and 30 min under optimized substrate loading 4 %, with 0.1 N NaOH at 50 °C. The graphical representation of the results can be seen in Figure (2). The maximum (TRS) obtained was 1.54 g/l when substrate was treated for 25 min. From the results obtained it is observed that, there was an increase in the (TRS) content up to 25 min and thereafter there was not much increase, therefore we chose time 25 min as optimum time.

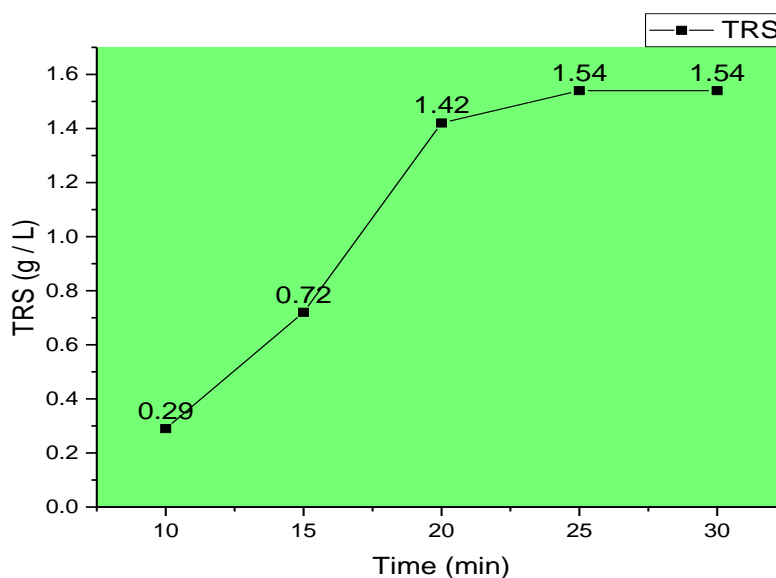


Fig. (2): Effect of pretreatment time on TRS

#### Effect of pretreatment temperature

Pretreatment of waste paper was carried out with different pretreatment temperature 30, 40, 50, 60 and 70°C under optimized substrate loading 4 % w/v and time 25 min with 0.1 N NaOH. The graphical representation of the results can be seen in Figure (3). The maximum (TRS) obtained was 1.89 g/l at 60°C. An initial increasing of (TRS) formation was observed when the pretreatment temperature increased from 30 to 60°C, at 70°C there was slightly decrease in the concentration of reducing sugar because of sugar degradation due to high temperature with relatively long time 70°C for 25 min, therefore 60°C was the optimum temperature.

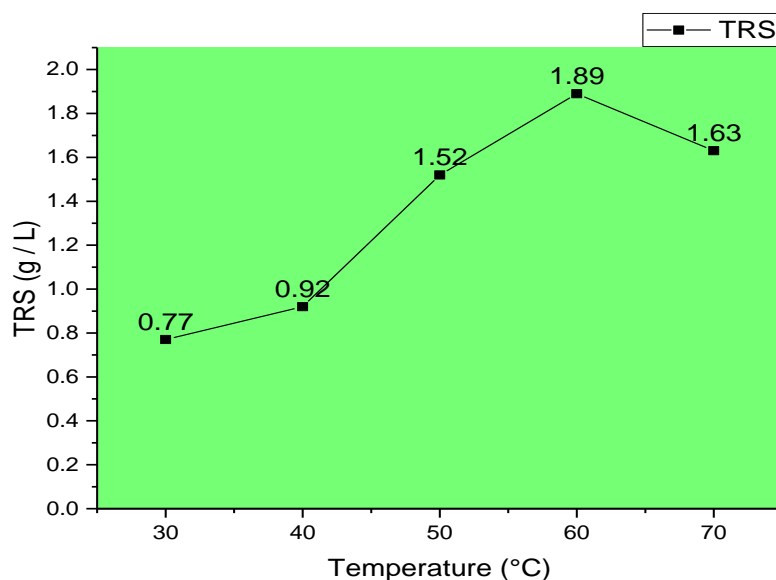


Fig. (3): Effect of pretreatment temperature on TRS

#### Hydrolysis of the pretreated waste paper

The slurry provided to the enzymatic hydrolysis had a (TRS) concentration of approximately 1.89 g/L after pretreatment. Enzymatic hydrolysis was performed at 40°C with pH adjusted to 5 for 48h. The maximum reducing sugars and hydrolysis efficiency percentage were 20.92 g/L and 78.4% respectively as shown in Figure

(4). In the current study, crude enzymes secreted from bacteria were efficiently utilized for hydrolysis instead of using commercial enzymes, in order to decrease the cost of bioethanol production.

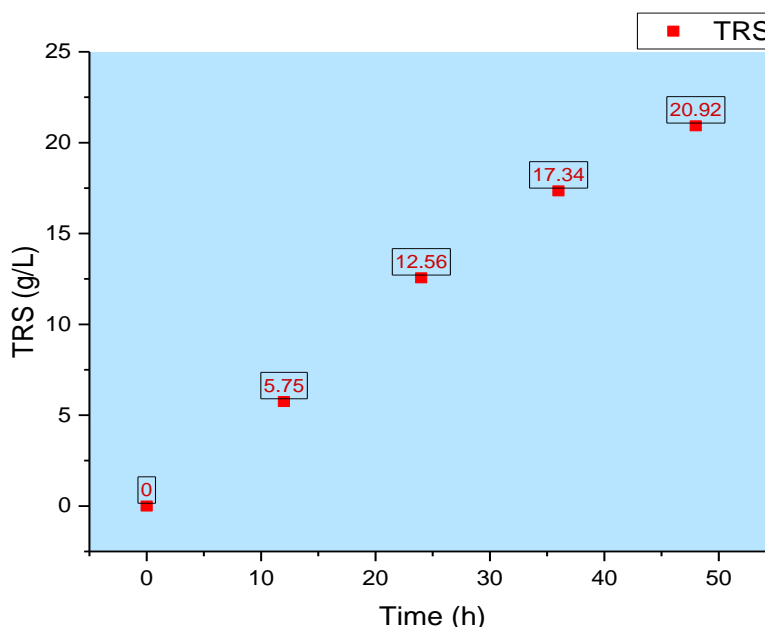


Fig. (4): Reducing sugar concentrations after enzymatic hydrolysis

#### Fermentation of waste paper hydrolysate

Fermentation of optimized hydrolysis hydrolysate which contained 20.92 g/L reducing sugar was performed using yeast *S. cerevisiae*. The maximum concentration of bioethanol estimated using (HPLC) was 9.5 g/L after 48h incubation, the yield and volumetric productivity were 0.454 g/ g glucose and 0.2 g bioethanol/L h respectively. Conversion efficiency can be found by assuming 1 g glucose will produce 0.511g bioethanol [23]. Hence, conversion (%) of fermentation process was 88.8%. The graphical representation of the results of consumption of reducing sugar during fermentation and the bioethanol produced are shown in Figure (5). The bioethanol production was found to increase quickly during the first 24 h of fermentation. It decreased only after reaching a maximum concentration of 9.5 g/L after 48h incubation specifying no benefit in extending fermentation time. The prolonged fermentation process caused reduction in bioethanol concentration after achieving the maximum yield; this may be happened because yeast *S. cerevisiae* will use accumulated bioethanol as a carbon source for its growth when the concentration of sugar started to exhaust. The bioethanol production, substrate type, pretreatment method, hydrolysis method and fermentation process conditions was compared to the previous studies Table 1. The bioethanol yield obtained in this study was similar to previous studies [6,8,25]. The variations in the results with different studies are likely due to the different pretreatment, hydrolysis and fermentation methods used in the bioethanol production [26,27]. On the other hand, the microorganisms type used in the hydrolysis and fermentation step can also affect the final bioethanol yield [28] [15].

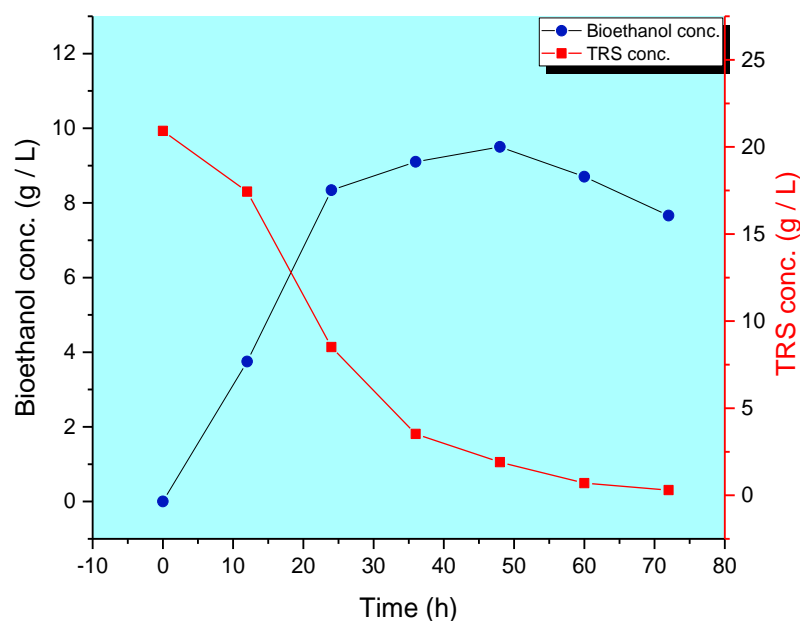


Fig. (5): Bioethanol production and sugar consumption during fermentation process

Table (10): Comparison of different bioethanol production process

Substrate	Pretreatment	Hydrolysis	Fermentation conditions	Bioethanol produced	Reference
Waste paper	Pretreatment with steam explosion	Enzymatic hydrolysis (crude enzyme)	SHF, <i>S. cerevisiae</i>	12.5 g/L (48h)	[8]
Waste paper	Dilute acid pretreatment	Acid hydrolysis	SHF, <i>Pichia stipitis</i>	3.73 g/L (24h)	[15]
Waste newspaper	US- alkaline	US-assisted enzymatic hydrolysis (commercial enzyme)	SHF, <i>S. cerevisiae</i>	14.1 g/L (48h)	[25]
Waste office paper	Acid pretreatment	Enzymatic hydrolysis (commercial enzyme)	SHF, <i>Spathaspora passalidarum</i>	0.42 g/g glucose (24 h)	[26]
Waste paper	Grinding using a kitchen blender	Enzymatic hydrolysis (commercial enzyme)	SSF, <i>S. cerevisiae</i> <i>Pichia kudriavzevii</i>	17.9 g/L 14.67 g/L (96 h)	[6]
Waste paper	Ultrasound assisted alkaline	Enzymatic hydrolysis (crude enzyme)	SHF, <i>S. cerevisiae</i>	9.5 g/L (48 h)	This study

## Conclusion

Waste paper was used as lignocellulosic biomass for the production of reducing sugars. The current study introduces optimization of ultrasound assisted alkaline pretreatment process to remove the most recalcitrant part of waste paper (i.e., lignin). Ultrasound assisted alkaline pretreatment process reduces the operation time of alkaline pretreatment process and expands the quality and recovery of pretreated lignocellulosic feedstock. The conversion of the reducing sugars to bioethanol was carried out using the separate hydrolysis and fermentation (SHF). Crude enzyme produced by *Cellulomonas uda* (PTCC) has proved his effectiveness in the enzymatic hydrolysis of waste paper. Sugars released from waste paper were subsequently fermented into bioethanol with *S. cerevisiae*. Bioethanol yields resulting from the waste paper using ultrasound pretreatment can display the effectiveness of this technique together with (SHF) process in the biofuel production.

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