Glucose production from Iraqi date-palm empty fruit fibers

Mohammad F. Abid[a,*], Yasser H.Sabah[b], Bilal A. Hameed[c], Farah T. Jasim [d]

[a]*Department of Medical Instrumentation Engineering, Al-Hikma University College, Baghdad, email: mohammad.abid@hiuc.edu.iq*

[b]*Department of Medical Laboratory techniques , Al-Hikma University College, Baghdad, Iraq, email:* yasser.hussam@hiuc.edu.iq

[c]*Department of Medical Laboratory techniques , Al-Hikma University College, Baghdad, Iraq, email:* belal.lateif@hiuc.edu.iq

[d]*Department of Chemical Engineering, University of Technology, Baghdad, Iraq, email: falsudany@yahoo.com*

Abstract:

In the present work, a simple technique was adopted to investigate the feasibility of producing glucose from the empty date fibers (EDF) fibers. Effect of the operating parameters such as type of acid, acid concentration, temperature, particle size, and reaction time on the yield of glucose (S) from the EDB fibers was studied. DESIGN-EXPERT 10.0.6.0 software was used for analysis and optimizes the operating variables. For the hydrolysis using hydrochloric acid, the maximum predicted sugar yield was 62.2% of the initial weight under optimal conditions: acid concentration of 11.0%, the temperature of 70°C, a reaction time of 50 min and an average particle size of 0.2mm. At the same operating parameters, the maximum predicted glucose yield using sulfuric acid was 41.7% of the initial weight. It was found that pretreatment of EDF fibers with 0.5M sodium hydroxide at optimum temperature increased the conversion of lignocellulose fibers by 20-25 %.

Keywords: Glucose; date-palm; lignocellulose fibers; acid hydrolysis

Introduction

Millions of producing palm-dates trees are growing in Iraq. However, the residue of the palm tree, especially the empty date bunch (EDB) has still not been completely utilized. Umikalsom et al. (1997) reported that the fresh fruit bunch of the Malaysian palm-oil contains about 49 wt% being the fruit whereas the other portion left was the solid wastes containing 24 % empty bunch, 14-15 % fiber, 6-7 % nucleus and 6-7 % shell. The EFB has a higher percentage of cellulose (~ 51% w/w) comparing to other biomass origins such as sugar cane bagasse, rice straw, and corn cobs (Rodríguez et al., 2003; Rivas et al., 2002). Table 1 shows the lignocellulosic ingredients of various origins of biomass, among them; EFB has the highest percentage of cellulose.

Table 1 Ingredient of different of lingnocellulsic sources.

Source	Composition (% by dry weight)					Reference
	Cellulose	Xylene	Hemicellulose	Lignin	Ash	
Malaysian Palm-Oil Empty Fruit Bunch	50.4	------	21.9	10.0	0.5	Umikalsom et al., 1997
Sugar Cane Bagasse	38.9	20.6	-------	23.9	------	Rodríguez et al., 2003
Corn Cobs	31.7	-------	34.7	20.3	------	Rivas et al.,2002
Rice Straw	43.2	20.2	26.0	10.2	0.4	Roberto et al, 2003

The EFB fibers contain alternating phases of highly ordered (crystalline) and disorderly arranged (amorphous) cellulose implanted in a matrix of hemicellulose (Sarkanen and Ludwig, 1971). This latter carbohydrate (also known as pentosane), constituting 20%-50% of the plant dry weight, is a branched polymer of pentose sugars, $(C_5H_{10}O_4)_n$. The fractions of cellulosic and hemicellulose are covered with an amorphous layer of lignin. Lignin with ingredients of 15%-25% of the plant material is a complex three-dimensional polymer consisted of carbon-carbon or ether bonding between phenyl-propane units (Millett et al., 1976). Many researchers (Fox et al., 1987) of the field reported that sugars could be extracted from cellulosic and hemicellulose materials with different steps. Pretreatments are designed to open the structure of lignocellulosic biomass prior to enzyme hydrolysis, to allow efficient production of C5 and C6 sugars. Hydrolysis of the major component, cellulose, has received the most attention, as it can be used to produce ethanol by fermentation. Cellulose exists in nature as a compact and complex matrix with lignin and hemicellulose (Côté, 1982). The cellulose is highly ordered and crystalline with amorphous regions. It is surrounded by lignin which acts as a physical barrier (Fox, 1987) and is associated with the hemicellulose. The lowering of the crystallinity of cellulose and elimination of lignin and hemicellulose are paramount objectives for any pretreatment

operation [9](Wu, 1997). Lignocellulosic biomass can be quashed by chipping, grind, or milling. The objective of mechanical crunching is to minimize the particle sizes of the biomass, as enhanced surface area leads to advance cellulose hydrolysis. The crystalline structure of cellulose is also broken utilizing these methods, though the amount varies according to the type of biomass and power applied in grinding. A ball mill is the most tool for efficacious breakage of the crystalline structure of cellulose (Millet, 1976). Acid treatments normally aim for high yields of sugars from lignocellulosic biomass (Côté, 1982). Acid usually has a greater effect on the hemicellulose and lignin than doe's alkali (Fox, 1987). There are many methods for acid treatments including the use of phosphoric acid (Ramos, 1996), sulfuric acid (Côté, 1982; Camacho, 1996), hydrochloric acid (Côté, 1982), or peracetic acid (Sakai, 1966). Reacting biomass with dilute sulfuric acid alters the crystalline nature of the cellulose structure by expanding the surface area of the biomass, allowing water penetration into the crystalline structure. Dilute sulfuric acid treatment improves the ease of solubilization of biomass and the formation of glucose (Millet, 1986; Tsao, 1978). Concentrated sulfuric acid pretreatment solubilizes cellulose by breaking down the hydrogen bonds (Camacho, 1996). According to Lai (1968), peracetic acid can also be used to treat lignocellulosic biomass. Peracetic acid is a powerful oxidizing agent that removes lignin, in a manner similar to ozone treatment. It can also cleave the aromatic molecules in lignin. Alkali treatment reduces the lignin and hemicellulose content in biomass, increases the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose and lignin-carbohydrate (Gratzl, 2000; Fox, 1987; Tarkow, 1969). Dilute sodium hydroxide is usually used for alkali treatment (Fan, 1987). Hydrogen peroxide can enhance the hydrolysis of the biomass by generating hydroxyl radicals and superoxides that attack organic materials. Azzam (1989) reported that about half of lignin and most hemicellulose in the biomass were solubilized with hydrogen

peroxide. Temperature and acid concentration were considered the main effective parameters of the hydrolysis reaction (Aguilar et al., 2002). Maximum conversion is achieved by applying high temperature, though product contamination with the presence of soluble derivatives such as furfural and hydroxyl methyl furfural, respectively (Rodriguez, 2003). Fengel and Wegener (1984) indicated that the mechanisms of acid hydrolysis of cellulose were leading to a scission of glycosidied which initially catalyzed by the action of the proton (H+) existing in the aqueous medium. Lavark et al. (2002) studied the effect of the ratio of liquid to solid on biomass conversion. They concluded that as the solid to liquid ratio was reduced, the rate of decomposition of xylose would also be reduced due to the dilution of the reaction slurry. In Iraq, researches on the extraction of glucose from the empty date palm bunch are scarce. The present work is aimed to investigate the effect of hydrolysis parameters, including the type of acid, acid concentration, temperature, particle size, and reaction time, on the glucose (S) yield from the Iraqi empty date palm bunch (IEDB).

Materials and methods

Analytical grade chemicals used during the experimental course are presented in Table.2.

Table (2) Chemicals used in the present work

Chemical	Specification	State	Supplier
HCl	36%, Clear colorless to light-yellow liquid	Liquid	BDH, England
H_2SO_4	98%	Liquid	Merck Inc.(Germany)
NaOH	99.8%	solid flakes	Sigma Aldrich
DNS reagent	3, 5-dinitrosalicylic acid	Powder	

The EDB fibers were washed with water and dried in an oven at 80°C for 12 h. After then, fibers were ground using a ball mill and sieved into three average sizes of particles (0.2, 0.4, and 0.8 mm). Each portion of the sieved- particle size was divided into four portions. The

first portion was acid hydrolyzed within a 250 mL flask using 5 to 15 wt % HCl with an increment of 2.5 wt% at a variable temperature of (40 to 80°C) with an increment of 10°C. The second portion was treated with 5 to 15 wt % H_2SO_4 at the same conditions as the first portion. The third and fourth portions were pretreated using a 0.5 M sodium hydroxide solution. The impregnation of the alkaline solution was implemented by a heat treatment in a boiling water bath for 1 hr. The pretreated samples were washed several times with distilled water and then dried prior to acid hydrolysis with HCl and H_2SO_4, respectively. The hydrolysis procedure was carried out into a 250mL flasks placed in a temperature-controlled oven. A Sample was taken every 15 min, the hydrolysate was collected and neutralized with a sodium hydroxide solution to a pH of 7. It was then further diluted with distilled water (1/10 v/v) and filtered through a 0.45 μm cellulose membrane before being analyzed.

Analytical procedure

The elemental analyzer of Perkin Elmer Model 2400 was used for the determination of the fiber composition. The total glucose produced in the hydrolysate was analyzed by the colorimetric technique. The color tests were made with 3-mL of reagent added to a 3-mL of glucose solution in 14-mm tubes. The mixtures were heated 5 minutes in a boiling water bath and then cooled under running tap water adjusted to ambient temperature. Cooling to ambient temperature was necessary by the effect of temperature on the absorbance of the colored reaction product. The color intensities were measured using the UV-Vis spectrophotometer (Model Cecil 1000) at 540nm with a slit width of 0.06mm using a reducing chemical reagent, 3, 5-dinitrosalicylic acid (Miller, 1959). Figure.1 illustrates the effect of glucose on light absorbency with variable wt% of DNS.

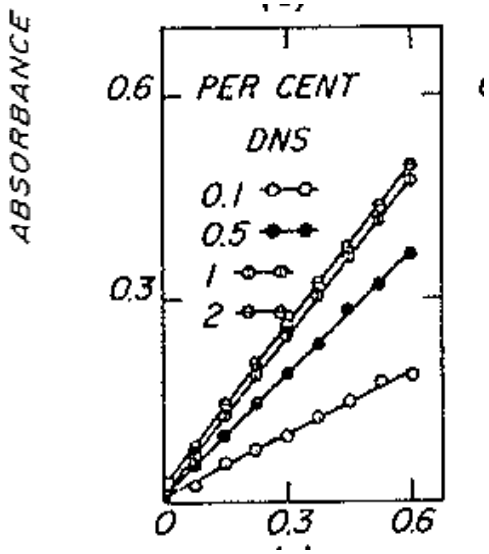


Fig.1 Variation of color produced with glucose and DNS (Miller, 1959)

The concentration of each compound in the liquid phase was determined using calibration curves obtained by analyzing standard solutions with known concentrations. The calibration curve for reducing sugar using HPLC is shown in Fig. 2

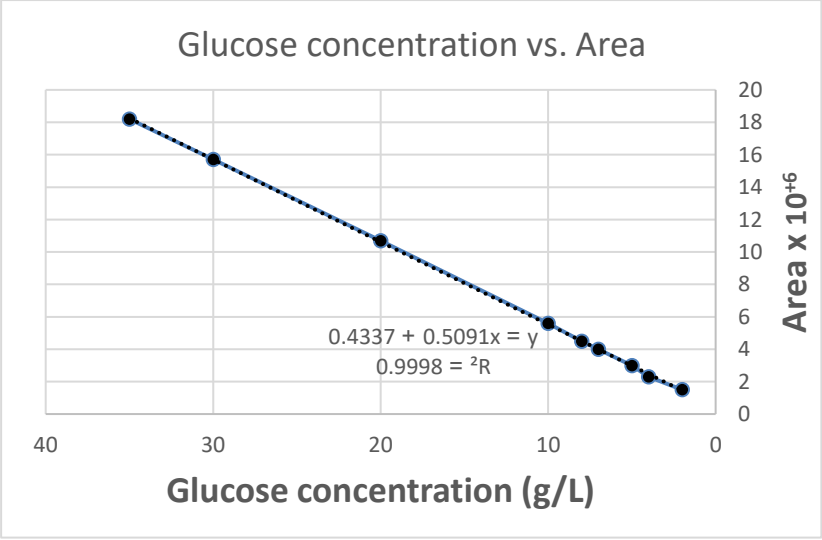


Figure 2: Calibration curve of glucose using UV-Vis (Model Cecil 1000)

Experimental design

In the present work, the factorial design method was used for planning the experiments because of its reliability in finding out the effects and interactions between the controlled

variables of the operating system (Montgomery, 2019). According to the method, the total number of experiments (N) is estimated by equation (1),

$$N=L^F \quad (1)$$

Where, F: number of controlled variables (i. e, factors), and L: number of levels of each factor. Table 1 lists the levels and operating factors of the present work. According to equation (1), the total number of experiments is 125.

Table 3 Levels and operating factors

Factors / level	Acid concentration, wt%	Temperature, ^oC	Time, min
1	3.0	40	5
2	7.0	50	20
3	11.0	60	35
4	15.0	70	50
5	19.0	80	75

Results and discussion

Table 4 lists the weight composition of the Iraqi fiber analyzed by Elemental Analyzer, Perkin Elmer Model 2400.

Table 4 Weight composition of the Iraqi EDB fiber

Source	Composition (% by dry weight)					Reference
	Cellulose	Xylene	Hemicellulose	Lignin	Ash	
EDB fibers	51.21	18.5	20.29	9.53	0.47	Ibn-Sina Research center

Effect of Operating Temperature

To investigate the influence of temperature on glucose yield a temperature range of 40 to 80°C was utilized. Figure 3 illustrates the variation of glucose yield as a function of processing time at different temperatures while other parameters were kept unchanged at

10%w/w solid, the average particle size of 200µm and 11% HCl. As can be seen in Figure 3, that after 5 min of operation the glucose yield was 7.5, 16, 16.5, 19, and 23 g/L at a temperature of 40, 50, 60, and 80 ^{o}C, respectively. While at 50 min of operation the glucose yield was 20, 25, 25, 28, and 30 g/L at 40, 50, 60, and 80 ^{o}C, respectively which meaning that temperature has a positive impact on glucose yield. This behavior could be attributed to the specific reaction rate constant (k) which increases, according to Arrhenius equation ($k = k_o\, e^{-(E/RT)}$), as temperature increased. However, as can be seen in Figure 6 that at elevated temperatures (70 to 80 ^{o}C) as processing time increased more than 50 min a decrease in glucose production taking place. This may be due to the possibility of decomposition of the reducing sugar to furfural and hydroxyl methyl furfural according to equation (1) Abdul Aziz et al. (2003).

$$\mathbf{C_5O_5H_{10}} + \text{Excessive Heating} \longrightarrow C_5O_2H_4 + 3H_2O \quad (1)$$

Xylose Furfural

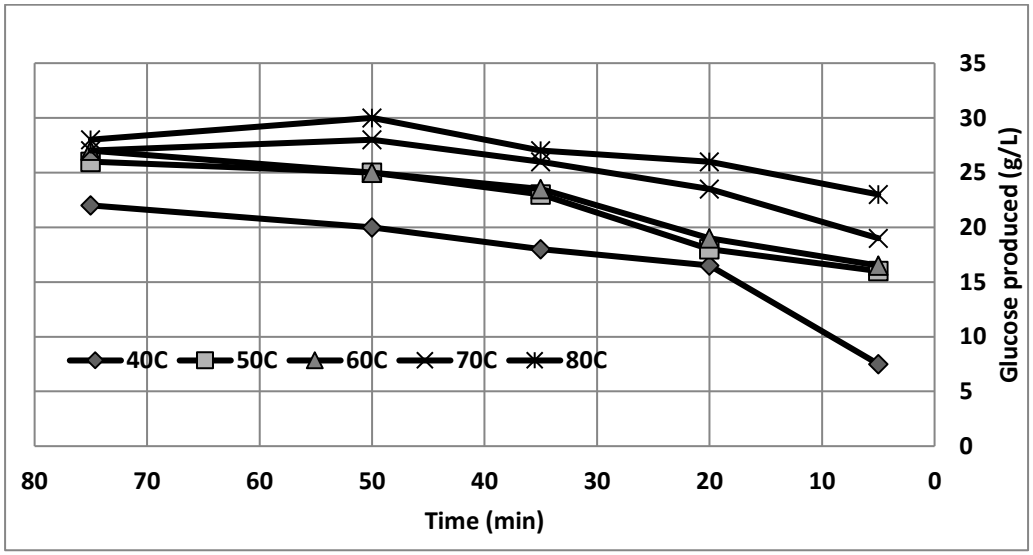


Fig.3 Variation of Glucose produced against time at different Temperature (11wt%HCl and 10% solid)

Effect of acid concentration

The influence of acid concentration on glucose yield is illustrated in Figure 4, where the produced glucose was plotted as a function of reaction time using different HCl concentration. Other operating parameters were kept unchanged at 200 µm average particle size, 60 oC, and 10 %w/w solid. As can be seen in Figure 4, that after 5 min of operation the glucose yield was 4.5, 12, 16.5, 17.5, and 19 g/L at HCl concentration of 3, 7, 11, 15 and 19%, respectively. While at 50 min of operation the glucose yield was 13, 22, 25, 26, and 27 g/L at 3, 7, 11, 15 and 19% w/w HCl, respectively. From these results, it could be concluded that the glucose yield was increased with an increase in the acid concentration, that the performance of acid hydrolysis was proportionally affected by the H+ concentration. Our results well agree with the findings of (Mosier, 2005) who studied experimentally the effect of acid hydrolysis on cellulose. He reported that the more hydrogen ions formed in the solution, the more rapid the cellulose fraction would be disrupted and glucose would be generated. It is also shown in Figure 4 that the rate of glucose production was decreased slowly as the processing time increased beyond 40 min. This behavior may be attributed o the concentration of hemilcellouse and xylose which decreased continuously with the acidification process

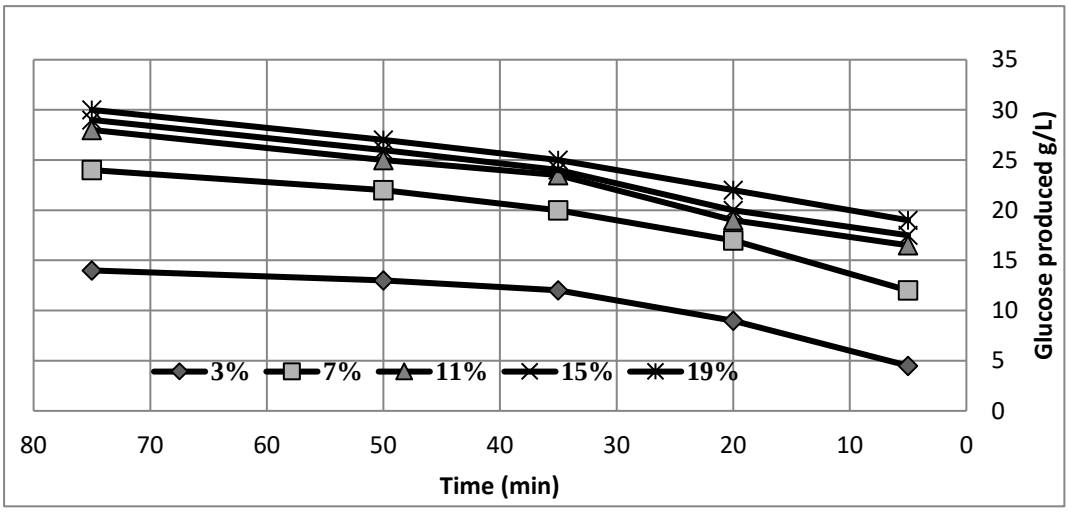


Figure 4 Variation of Glucose produced against time at different HCl concentration (60^{o}C, and 10% solid)

Effect of Pretreatment with NaOH

The effect of treatment by NaOH on glucose yield is tabulated in Table 5. Results indicated that the hydrolysis of the pretreated fibers resulted in about 20-30 percent more glucose at 50^{o}C and 50 min with 0.2 mm average particle size. In fact, the glucose concentration was more observed during the hydrolysis after pretreatment by 0.5M of NaOH solution. Fig. 5 illustrates the variation of glucose produced vs. time for solid particles treated and untreated with NaOH at (11%w/w, 50 ^{o}C, and 0.2 mm average particle size). As can be seen, after 5, 20, 35, 50 and 75 min, the glucose production was 16.5, 19, 23.5, 25, and 26 g/L respectively for treated with NaOH while for untreated produced glucose was 12, 13, 15.5, 17, and 19 g/L. Consequently, glucose produced after NaOH treatment is predominant over that produced without treatment.

Table 5 Effects of NaOH treatment, average particle sizes, and acid type on average yields of glucose from acid hydrolysis at (50^{o}C and 50 min.)

Average size of fiber, mm	Yield of glucose g/L			
	Hydrolysis with 11.0% HCl		Hydrolysis with 11.0% H_2SO_4	
	Treated with NaOH	Untreated with NaOH	Treated with NaOH	Untreated with NaOH
0.2	25.3	17.0	19.4	11
0.4	22.1	13.2	14.9	8.7
0.8	13.7	7.8	8.9	5.2

The hydrolysis reaction was found to be faster with treated cellulose fibers by NaOH. The present results confirmed the findings of (Lim et al., 1996) who studied the influence of NaOH treatment on the production of reduced sugar from acid hydrolysis of cellulose. He suggested that lignin might be removed after NaOH treatment leaving carbohydrate fraction reactive towards the acid action. The alkaline pretreatment of empty EDB fibers was

believed to modify the physical structure of lignin while the NaOH solution acted as an effective intra-crystalline swelling agent.

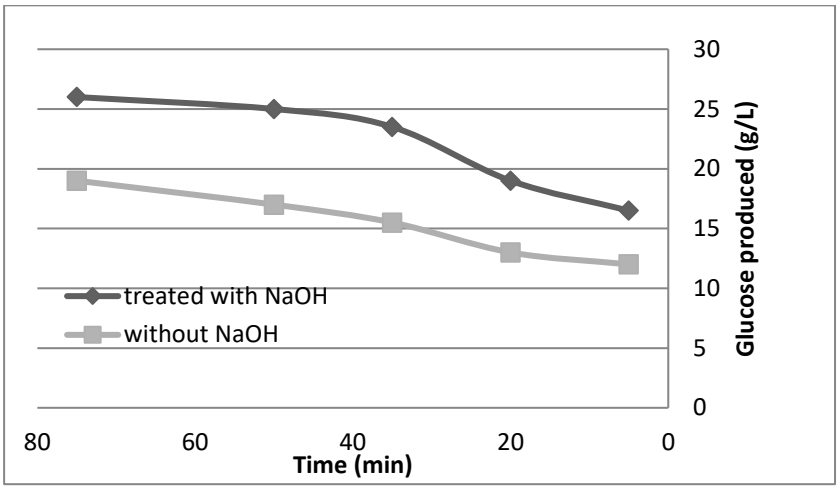


Fig. 5 Variation of Glucose produced vs. time for solid particles treated and untreated with NaOH at (11%w/w, 50 ^{o}C, and 0.2 mm average particle size)

Effect of particle size

Figure 6 illustrates the influence of the average particle size on glucose yield. Three different sizes of solid particles (0.2-0.8mm) were studied. As can be seen, the effect of smaller average particle size on glucose yield was pronounced more than that of other particle sizes. This may be attributed to the specific surface area of particles which was greater for the smaller particle. This surface area secures a higher reaction rate between the EDB fibers and acid. Moreover, for solid particles with a size bigger than 0.2mm, the penetration of acid was slower and the sugar glucose concentration gradually decreased as shown in Figure 6. This was due to large particle size and insufficient contact time for the acid to penetrate inside the large solid particles.

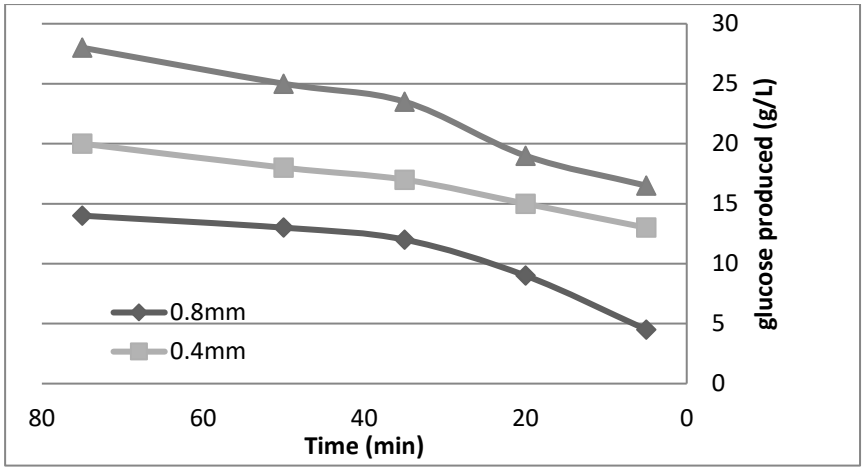


Figure 6 Variation of glucose produced at different average particle size

Effect of acid types on glucose product

DESIGN-EXPERT 10.0.6.0 software (Stat-Ease Inc**.,** 2000) was applied for analysis and optimizes the studied operating conditions for EDB treatment by HCl. It was found that acidification of EDB fibers at temperature= 70 ^{o}C, average particle size = 0.2 mm, HCl concentration = 11 %w/w, and operating time = 50 min gave the optimum glucose production. To estimate the glucose production with the treatment of EDB fibers by H_2SO_4, the same operating parameters were used. Figure 7 illustrates the Effect of acid type on glucose production at optimum operating parameters. It is clearly observed that the hydrolysis using HCl gives a maximum sugar yield of 62.2% of the initial weight under optimal conditions. However, at the same operating parameters, the maximum estimated glucose yield using sulfuric acid was 41.7% of the initial weight.

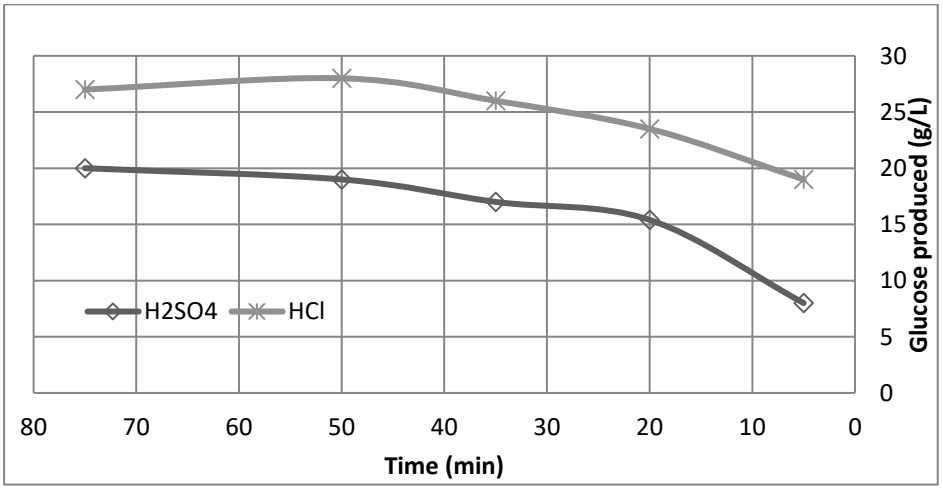
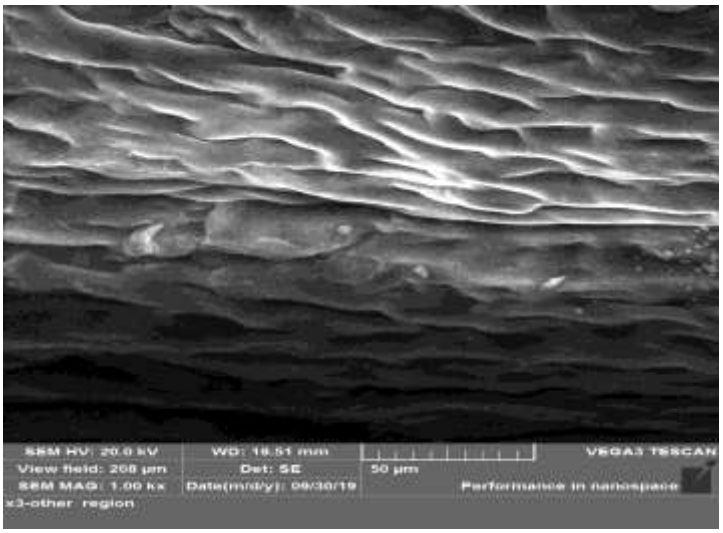


Figure 7 Effect of acid type on glucose production at optimum operating parameters (temperature= 70 ^{o}C, average particle size = 2 mm, acid concentration = 11 %w/w)

Studies on surface morphology

Figure 8 (a, and b) images the SEM snapshots of EDB fibers. The shown surface morphology revealed that EDB fibers in Fig. 8b, which treated with NaOH has more active sites than that of untreated fibers of Fig. 8a. The higher number of active sites, observed in Fig. 8b, revealed the enhancement in the reaction rate of acid hydrolysis resulting in a higher yield of glucose (see Fig. 5). The uniform distribution of active sites shown in Fig. 8b proved the perfection of the acid hydrolysis process. EDS images for untreated and treated EDB fibers with NaOH are shown in Figs. 9a and 9b respectively. In addition Table 3 lists the elemental analysis for the untreated and treated fibers with NaOH. The observation of Figure 9 revealed the effect of NaOH on enhancing the number of free active sites required for cellulose reaction by scavenging the impurities (e.g., K, S, and Cl) deposited on the fiber surface. Moreover oxygen element connected to the sites of cellulose was reduced from 37.0 to 19.8wt%. Consequently, the carbon element rose to 76.3wt% which increased the rate of acid hydrolysis.

(8a)



(8b)

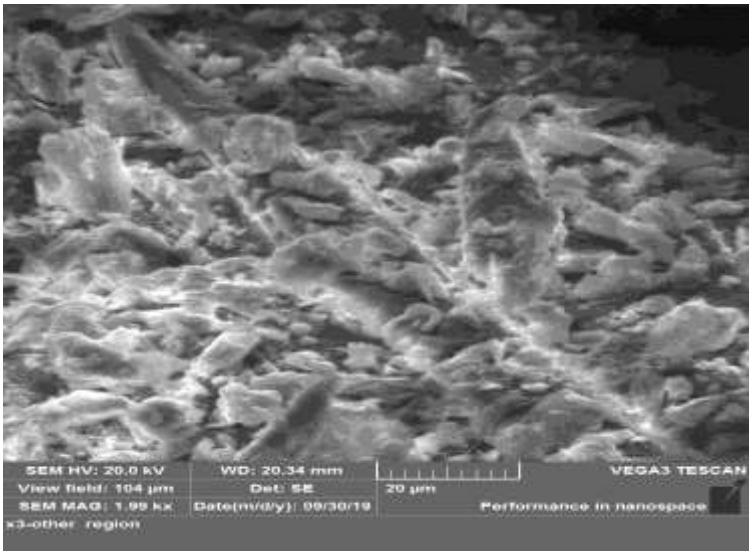


Figure 8: SEM images of untreated fibers (a) and treated fibers (b) with NaOH

(9a)

(9b)

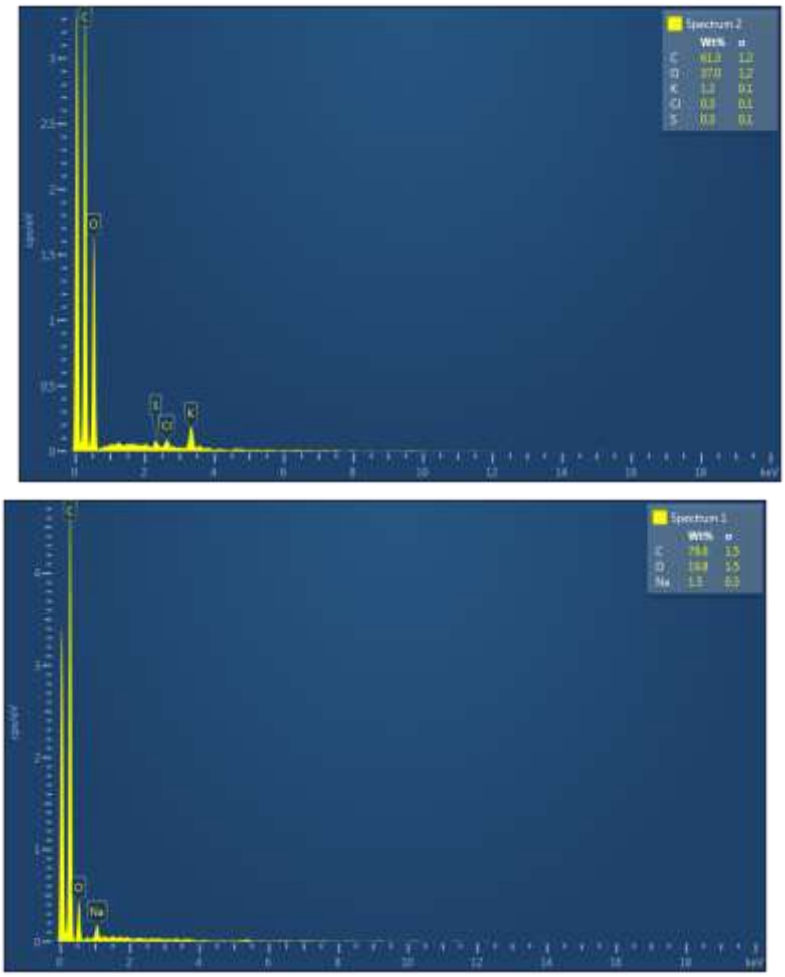


Figure 9: EDS images of (a) untreated fiber (b) treated fiber with NaOH

Table 3: Elemental analysis of untreated and treated EDB fibers with NaOH

Property	Elemental analysis, wt%					
	C	O	K	Cl	S	Na
Untreated fibers	61.3	37.0	1.2	0.3	0.3	0.0
Treated fibers	78.6	19.8	0.0	0.0	0.0	1.5

Conclusion

In the present work a simple and feasible method was applied to investigate the effect of the hydrolysis parameters, including acid concentration, temperature, particle size, and reaction time, on the yield of the glucose (S) from the (IEDB). DESIGN-EXPERT 10.0.6.0 software was used for analysis and optimizes the studied operating conditions. For the hydrolysis using HCl, the maximum estimated sugar yield was 62.2% of the initial weight under optimal conditions: acid concentration of 11.0%, the temperature of 70°C, a reaction time of 50 min and an average particle size of 0.2mm. At the same operating parameters, the maximum estimated glucose yield using sulfuric acid was 41.7% of the initial weight. It was found that pretreatment of EDB fibers with 0.5M NaOH at optimum temperature increased the conversion of lignocellulose fibers by 20-25 %.

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