Production of Biofuels from Selected Cellulosic Waste materials

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ABSTRACT

In this study four types of cellulose-rich municipal solid wastes (residuals) of orange, banana peel, corn residues, and saw dust were used as raw materials. These cellulosic substrates usually have a lot of lignin content which prevents the process of saccharification by microorganisms. Thus pretreatment methods of enzymatic, acid or base with enzymatic treatment and dilute acid followed by autoclaving were necessary to dignify these wastes and to obtain higher reducing sugar yields and hence higher ethanol production. Dilute HCl acid of 1% followed by autoclaving at 121℃ for 30 min proved to give good result where significant amounts of reducing sugars were obtained at the end of the saccharification process. Orange peel proved to give the highest glucose concentration of an average of 6000 mg/l on day 4 of the saccharification process. Fermentation was carried out for the hydrolyzed samples using Saccharomyces cerevisiae yeast. The amount of ethanol produced after fermentation was found to be the highest for orange peel having a value of 1300 mg/l after 96h of incubation. As science is proceeding, engineered microorganisms could help to produce sustainable fuels from cellulose-rich municipal solid wastes in the future.

Key Words: ethanol, biofuel, treatment of cellulose-rich municipal waste, fermentation, sustainable fuels.

انتاج وقود حيوي من نفايات سليلوزية

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الخلاصة

تم استخدام اربع انواع من المخلفات الصلبة المنزلية الغنية بالسليلوز والمتضمنة قشور البرتقال وقشور الموز والمخلفات النباتية المحيطة بعرنوس الذرة ونشارة الخشب كمواد اولية. غالبا ماتحتوي هذه المواد على كميات عالية من اللكنين الذي يعيق وصول الاحياء المجهرية الى مادة السليلوز واتمام عملية التسكير ، تم استخدام الانزيم والانزيم مع الحامض المخفف والحامض المخفف يتبعه معالجة حرارية كطرق معالجة ابتدائية ضرورية لتكسير اللكنين وانتاج نسبة اعلى من السكر المتبقي وبالتالي نسبة اعلى من الكحول. اثبتت النتائج ان استعمال حامض الهيدروكلوريك المخفف بنسبة 1% يتبعه معالجة حرارية بدرجة121م° ولمدة 30دقيقة انها افضل طريقة معالجة مقارنة مع الطرق اعلاه في انتاج سكر الكلوكوز اذ اعطى مسحوق قشر البرتقال اعلى نسبة تحول الى الكلوكوز6000 ملغم/لتر في اليوم الرابع من بدء عملية التسكير (Saccharification) . تم اجراء عملية التخمير لعينات محلول السكر الناتج عن تسكير مسحوق قشر البرتقال باستعمال الخميرة Saccharomyces cerevisiae وتم قياس تراكيز الكحول الناتج

ووجد ان اعلى نسبة تحول كانت بعد مرور 94 ساعة من بدء عملية التخمروبلغ 1300 ملغم/لتر وعليه فان هذه النَتائِج تشيرالى المستقبل الواعد لانتاج كحول الايثانول من النفايات السليلوزية الصلبة وعلى نطاق واسع.
الكلمات الرئيسية: الكحول، وقود حيوي، معالجة النفايات الصلبه البلدية الغنية بالسليلوز، التخمير، الوقود المستدام.

1. INTRODUCTION

In a world where the dumping of wastes is causing serious harm to the flora and fauna of the areas surrounding the dumping sites, the concept of using the wastes for production of energy forms a solution which is easily adoptable, cheap and efficient. One of the most abundant sources of energy in the world is the bio-polymer cellulose, which forms a major component of most plant and algal cell walls. The ability of organisms such as species of Trichoderma, Aspergillus, and Clostridium, etc. to produce the enzyme cellulose enables them to hydrolyze this cellulose into its constituent glucose units, **Taherzadeh, et al., 2007**. The glucose can then be utilized by organisms of the genera Saccharomyces which can ferment the glucose into alcohol such as ethanol, **Perlack, et al., 2005.**

Cellulose is the most abundant polysaccharide on earth and a major polymeric chain of glucose molecules. The cellulose molecule is comprised of long chains of cellobiose molecules joined together by β-1,4-glucosidic bonds. The molecular weight of the cellulose ranges from 300,000 to 500,000 (1,800 to 3,000glucose units) **Goyal, 2011**. Pure cellulose has a low solubility in water, and the molecules are tightly bound together by hydrogen bridges to form chains. Cellulose can break down to form glucose by either acid or enzymatic hydrolysis. One kilogram of cellulose is capable of being convened to 0.5 kg of glucose. This process is used by the ruminants (cows, deer, sheep, etc.) for the production of glucose, whereas other animals cannot digest cellulose and must ingest sugars directly, **Carere, et al., 2008**. One constraint to the production of cellulose is available land, because land that could be used for cellulose production may instead have to be used for other purposes, such as food production. On the other hand, the cellulose found in waste is a "free" source, as no additional land is needed for its production and no additional resources must be allocated, **Lynd et al., 2005**.

The reclamation of cellulose from wastes thus makes a great deal of sense, it does not compete with food production and it's available at low costs. In municipal solid waste (MSW) cellulose is found mostly in fruit rinds, paper, paper products, which constitute a major fraction of refuse. Wood and cotton are also sources of cellulose, but their fraction within the waste stream may be small. The production of ethanol from lignocellulosic biomass [corns over, wheat straw, sugarcane bagasse, rice straw, rice hull, corn cob, oat hull, corn fiber, woodchips and cottons talk; grass, alfalfa, and various weeds such as Saccharumspontaneum, Lantana camara, Eichhorniacrassipes (water hyacinth), etc.] has become one of the best alternatives, because these sources have widespread abundance and the cost of their procurement is relatively cheap, **Perlack, et al., 2005.**

1.1 Ethanol

Ethanol is an oxygenated fuel with high octane value like that of petroleum fuels. Ethanol is known to run combustion engines at higher compression ratios and thus provides superior performance, **Wheals, et al., 1999**.The blending of ethanol into petroleum-based automobile fuels can significantly decrease petroleum use and release of greenhouse gas emissions. Further, ethanol can be a safer alternative to the common additive, methyl tertiary butyl ether (MTBE), in gasoline. Thus, ethanol can be a substitute to mitigate the problems associated with the rising energy demands

across the world as well as a way to reduce greenhouse gas emissions to an extent of 85%, **Perlack, et al., 2005.** Ethanol can be produced either from petroleum products or from biomass, such as agriculture residues. The technological advances in recent years are promising to produce ethanol at low cost from lignocellulosic biomass knowing as bioethanol, **Aden, et al., 2002**. Bioethanol production from sugar cane and starch rich feed stocks such as corn, potato, fruits etc., is considered first generation process and it has already been developed. The long-term viability of this process is in question because it will require significantly increased amounts of cultivatable land and significant hike in food prices that will ultimately lead to food insecurity, **Mitchell, 2008**.

Estimates clearly point to the fact that first generation ethanol production process can not sufficiently meet the global energy needs. Therefore, second generation processes to produce bioethanol are gaining momentum. The second generation processes will use lignocellulosic materials for this purpose. Thus the objective of the present study is to investigate the possibility of bioethanol production from lignocellulosic materials relying on technologies that will efficiently hydrolyze cellulosic biomass to fermentable sugars.

1.2 Cellulosic Biomass and Their Sugar Composition

Lignocellulosic biomass consists of lignin, cellulose, hemicellulose, pectin and other components as shown in **Fig.1**. Cellulose is the principle component of lignocellulosic biomass and its concentration ranges from 40 to 50% of the dry weight **Fengel and Wegener, 1984.** Cellulose is from a family of organic compounds called polysaccharides, which are polymers of simple compounds called monosaccharaides, such as glucose. The cellulose molecules are bound together tightly by a large number of hydrogen bridges along the chain. The degree of polymerization and crystallinity of cellulose varies from species to another and this is shown to have a significant impact on hydrolytic process (acidic and enzymatic) **Zhang, et al., 2004**. Hemicellulose is less complex, its concentration in the lignocellulosic biomass is 25 to 35% and it is easily hydrolysable to fermentable sugars. Hemicellulose is a heteropolysaccharide composed of pentoses (D-xylose and D-arabinose), hexoses (Dmannose, D-glucose and D-galactose) and sugar acids **Saha, and Cotta, 2007**. Softwood hemicellulose mainly contains mannose as a major constituent whereas hardwoods mainly contain Xylans**Balan, et al., 2009.**

Lignin is the third major component of lignocellulosic biomass and its concentration ranges from 20 to 35% **Aden, et al., 2002**. It is a complex polymer of phenyl propane. Lignin acts as cementing agent and an impermeable barrier for enzymatic attack. Lignin provides plants with the structural support and impermeability they need as well as resistance against microbial attack and oxidative stress. These properties of lignin may be attributed to its amorphous nature, water insolubility and optical inactivity. The later properties also make it tough to degrade **Fengel, and Wegener, 1984.**

2. MATERILS AND METHODS

2.1 Experimental Work

All sets of experiments were conducted in a batch mode at room temperature unless mentioned. The experimental work was carried out in the laboratory of Graduate Studies in the Department of Environmental Engineering in College of Engineering and Department of Biotechnology in College of Science /University of Baghdad. Four types of cellulose-rich municipal solid wastes (residuals) were used as raw materials including orange and banana peels, corn residues, and saw dust.

Residuals were collected individually, weighed and dried at 110 C° for 18 h. in an air-drying oven (Hamilton).
Samples were ground into powder using food processer (Brown, China), sieved to obtain fine uniform powder. Dried powder samples were stored in sealed plastic bags at room temperature. Three different treatment methods as in below were investigated to determine the most efficient method for glucose production, and to determine the kind of sample containing maximum glucose concentration to be utilized for yeast fermentation.

2.1.1 Enzymatic hydrolysis

Enzymatic hydrolysis was determined according to the method described by Wilkins, **Wilkins, et al. 2007.** Ground residuals were added into 250 ml conical flasks containing 50 mM sodium acetate buffer at pH 4.5 to obtain 100 ml of sample/water slurry with solid fractions of 2, 4, 6, 8, and 10%. The slurries were hydrolyzed by using varying concentration of enzyme (2.0-5.0 ml) and with continuous shaking at incubation temperature of 30°C for 7 days**.** A sample of 10 ml was drawn each day, filtered and centrifuged at 1000 rpm for 30 min. to obtain clear supernatants. Two ml of the supernatant was mixed with 3 ml of DNS (3, 5-dinitrosalicylic acid) to determine the level of reducing sugar, heated for 15 min in a water bath. The DNS reagent will react with any sugar if present, producing a red-brown product. Samples were cooled to room temperature and 6 ml of distilled water was added to each and shacked well. One ml of Rochelle salt (4gof $CuSO_4.5H_2O$ hydrated copper sulphate with 24 g Na_2CO_3 sodium carbonate, 16g sodium potassium tartrate $NaK(CH_2OH)_2(COO)_2.4H_2O$ in 100 ml distilled water) was added to the supernatant to fix the color (red-brown) products before measuring the absorbance of the supernatants at 575 nm in an spectrophotometer instrument **Miller, 1959**. Glucose concentration was also measured by a glucose HK assay kit from Sigma at 505 nm.

2.1.2 Chemical and thermal hydrolysis

In chemical and thermal hydrolysis processes dilute hydrochloric acid of (1%) was used according to the method described by **Yang et al., 2002.** Samples with dilute HCl acid were mixed at a solid/liquid ratio of 2, 4, 6, 8, 10 g in 100ml for 15 min at 30°C. Followed by steam treatment undertaken in an autoclave at a constant temperature of 121°C for 30 min. Samples were filtered using textile fiber. A sample of 10 ml was drawn individually each day, filtered and centrifuged at 1000 rpm for 30 min. to obtain clear supernatants **Yang, et al., 2002.** Glucose concentration was also determined using the glucose HK assay kit. The same procedures above were repeated by using dilute sulfuric acid $H_2SO_4$of (1%) and sodium hydroxide NaOH as alternative chemical hydrolysate.

2.2 Microbial Fermentation

Fermentation of the reaction mixture containing reduced sugar was carried out for seven days of incubation at 30°C in an incubator shaker. A 5% of the yeast inoculum was added to the substrate for fermentation to convert simple sugars to alcohol using *Saccharomyces cerevisiae*. Sampling was done after 24, 48, 72, 96, 120, 144 and 168h of incubation. Ethanol concentration was determined by gas chromatography (GC) using Perkin Elmer Gas Chromatograph with a flame ionization detector and nitrogen as carrier gas. The GC was set at 150 ˚C as oven temperature, 200°C as injection temperature and 220°C as detection temperature. Standards were prepared by using 5ml

ethanol alcohol. Ethanol concentration was calculated based on the area under the peak for samples and standard solutions.

3. RESULTS AND DISCUSSION

3.1 Influence of Different Pretreatment Methods

The influence of different pretreatment methods on glucose and ethanol yields for four different municipal waste samples were investigated in the supernatant of the samples. **Fig.2** determines the most effective pretreatment process for saccharification, as in **Fig.2 process c** shows that the highest cellulose conversion to glucose was 10 mg/l of orange peel powder using dilute hydrochloric acid and thermal treatment as a pretreatment method. Results indicate that dilute HCl acid breaks most of the cellulose and hemicelluloses in the samples and leaves the cellulose fibers easily accessible for scarification after being autoclaved at 121℃ for 30 min. The highest yields of reducing sugars were observed to be 10 mg/l (glucose) for 10g of orange powder. **Fig.2** also revealed that enzyme and enzyme combined with acid pretreatment processes were found to produce lesser amounts of sugars (1.0 mg/l and 3.0 mg/l for 10g respectively for orange powder. Higher acid concentrations could be used to overcome low glucose yield, but dilute acids pretreatment is preferred for its easy operation, and comparatively low cost. **Fig.3** shows that saccharification is valid for different weights of orange peel powder and the values increased with increasing saccharification time and mostly peaked after 96 h of incubation (the fourth day). The peaking of glucose concentration at 96 h could be attributed to depletion of the hydrolysable polysaccharides. **Fig.3** revealed a maximum glucose concentration of 5800 mg/l for10g orange peel powder substrate which was selected for the subsequent experiments. Orange peel powder substrate was hydrolyzed using (HCl, H_2SO_4, NaOH) as pretreatment processes. Samples were analyzed each 24 h. Dilute HCl followed by autoclaving proved to yield the highest glucose concentration of 5650 mg/l for 10 g orange peel powder at 96 as shown in **Fig.4** .

Fig.5 ascertained the previous approval that dilute HCl acid also gave the highest glucose concentration of 6500 mg/l in other experience, dilute H_2SO_4 gave 5000 mg/l and NaOH gave 1000 mg/l at 96 h. For the dilute HCl hydrolysate medium, pH was investigated. It was 5.6 on day one, decreased slowly and remained above 5.4 throughout the initial 4 days of fermentation and then decreased rapidly from 5.4 to 4.7 after 4 days of cultivation. The pH behaved steadily around 5.0 during the entire fermentation process and did not affect the growth of yeast cells as shown in **Fig.6.**

3.2 Fermentation

A concentration of glucose of 6000 mg/l was fermented by *Saccharomyces cerevisiae*. **Fig.7** shows the time course of ethanol production as glucose from fermented orange peel powder. The glucose concentration decreased gradually from 6000 to 3000 mg/l through 4 days (96h) upon using dilute HCl. The ethanol production rate in the early phase of the culture was relatively slow but rapidly increased after 48h and peaked after 96 h of incubation and then slightly decreased, apparently due to ethanol oxidation to aldehydes and carboxylic acids as shown in **Fig.8.** Fermentation was completed after 96h, where ethanol reached a maximal concentration of 1300 mg/l. This indicates that the consumption of glucose by yeast cells was virtually in sync with the ethanol production. The ethanol yield was calculated according to the following equation:

$$\text{Ethanol consumption} = \frac{\text{Ethanol produced mg}}{\text{Remaining glucose mg}} \quad (1)$$

Ethanol consumption = (1300 mg)/(3000 mg) = 0.43 mg ethanol/mg glucose after 96h .
Which is substantially close to the ethanol yield (0.45 g ethanol/g glucose) reported by **Zhang, et al., 2004** and in agreement with those obtained from hydrolysis dry mass peel. Ethanol yield along the fermentation process is shown in **Fig.8**. The results suggest that *Saccharomyces cerevisiae* can grow well in the medium and can convert glucose to ethanol in convenient concentration.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Dilute acid followed by autoclaving proved to be the most efficient pretreatment methods to treat cellulose-rich municipal solid wastes (residuals) of orange peel and sawdust. Significant amounts of reducing sugars were obtained at the end of the saccharification process, using orange peel waste which proved to give the highest glucose concentration of 6000 mg/l on day 4. Fermentation was carried out for the hydrolyzed samples using *Saccharomyces cerevisiae*. The amount of ethanol produced after fermentation were analyzed by gas chromatograph and found to be the highest for the same fruit of 1300 mg/l and a yield value of 43% after 96h. The results indicate the promising future for generation of ethanol from cellulosic wastes on a large scale**.**

4.2 Recommendations

Future research may follow the suggested pathways:

1. Verification on a larger scale of the process is needed to demonstrate that the unit operations of enzyme, enzyme and acid, and acid hydrolysis will perform as anticipated on a larger-than-laboratory scale, as well as to determine what problems may occur during the operation of the integrated process.
2. Basic laboratory research is needed to improve the unit operations in order to improve process economics and develop an advanced strain of bacterium that could lead to more efficient fermentation of cellulosic materials and expand the amount of biomass material that can be converted into ethanol.
3. Suggesting pretreatment methods to improve ethanol production that should make the lignocelluloses available to the enzymatic attack, in order to obtain an efficient hydrolysis.
4. The data obtained in this research could be benchmarked against data presented for other type of food waste or bioreactor configurations such as pH, temperature, mass fraction or pretreatment acid concentration through the entire process.

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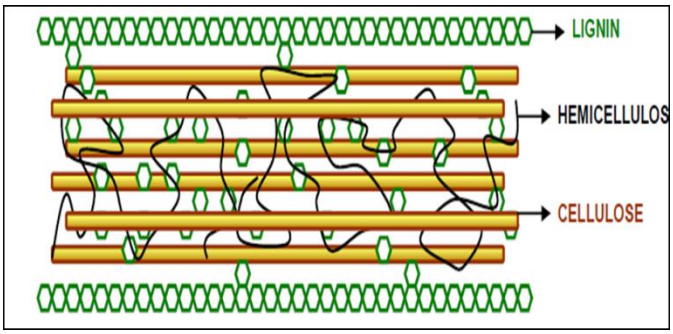

Figure 1. Representation oflignocellulosicstructure showing cellulose, hemicellulose and lignin fractions **(Fengel and Wegener, 1984) .**

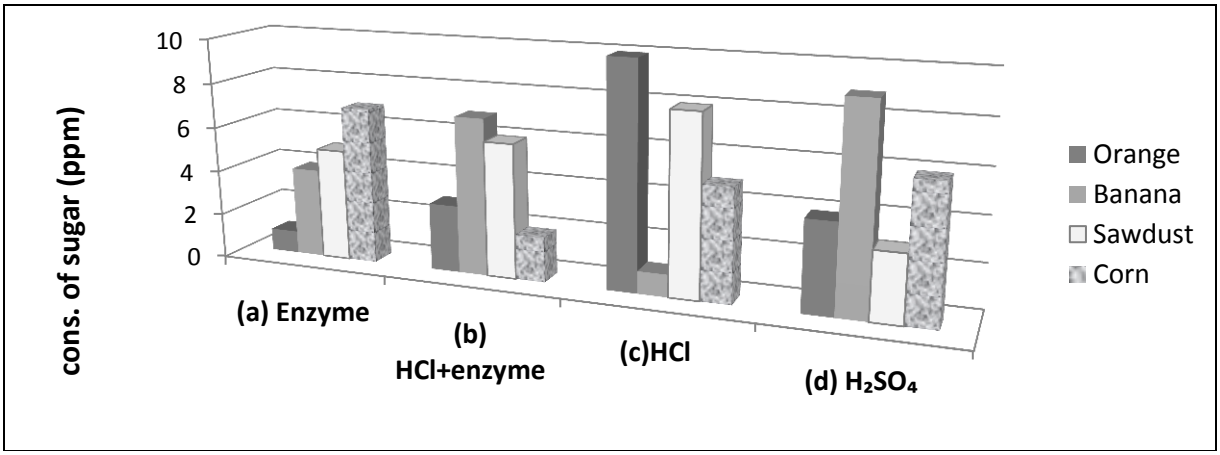

Figure 2. Concentration of reduced sugar after pretreatment with(a) enzyme (b) 1% HCl and enzyme (c) 1% dilute HCl followed by autoclaving at121℃ for 30 min
(d) 1% sulfuric acid followed by autoclaving at121℃ for 30 min

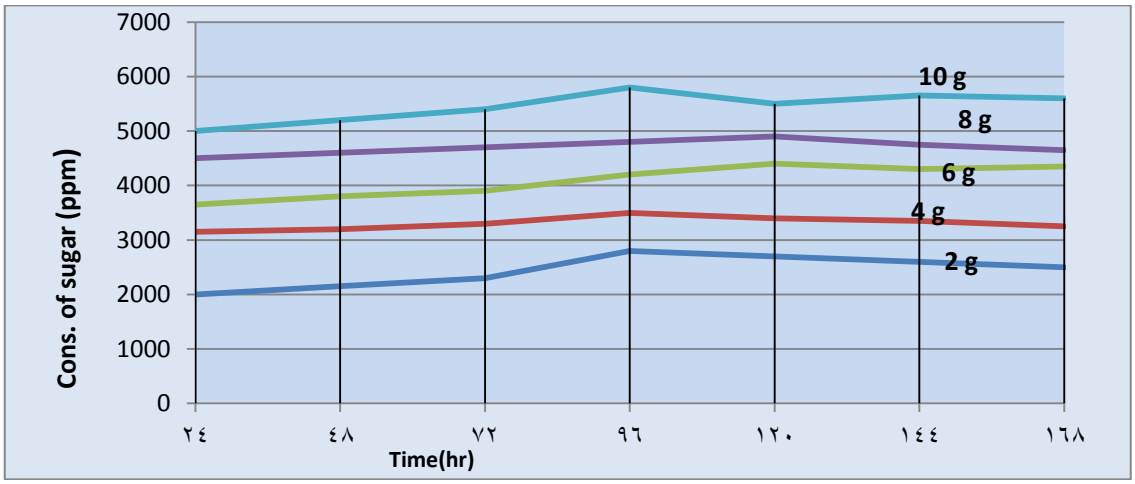

Figure 3. Time course of glucose production from lignocellulosic orange peel powder using 1% HCl followed by autoclave at 121℃ for 30 min.

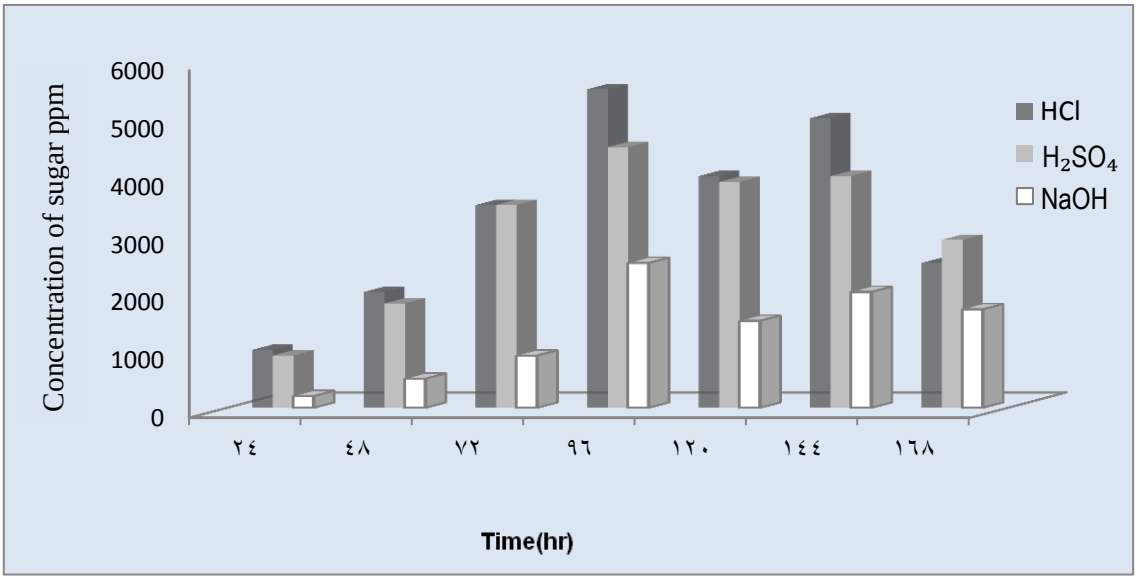

Figure 4. Time course of glucose production for 10g lignocellulosic orange peel powder using HCl, $H_2SO_4$and as hydrolysate.

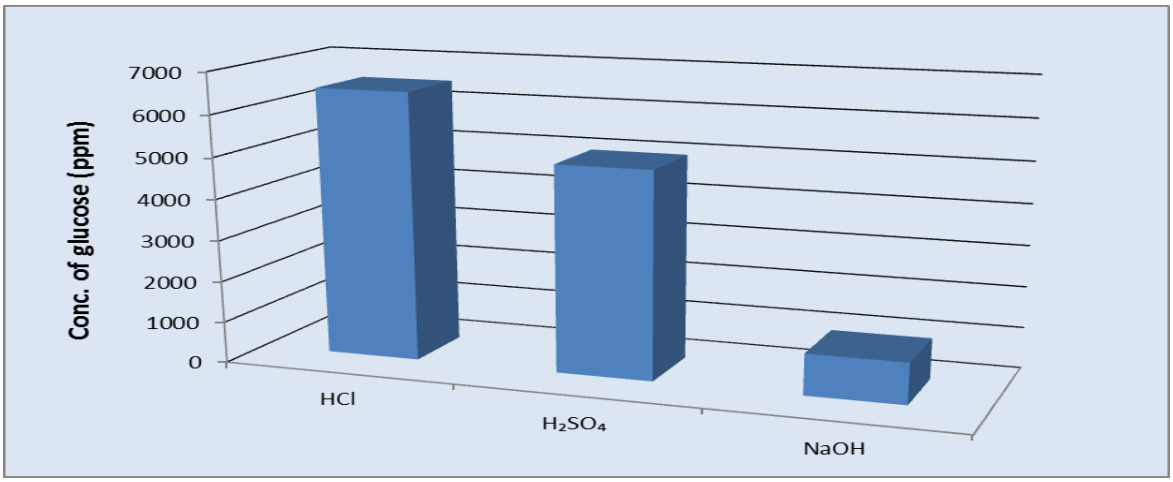

Figure 5. Glucose production using dilute acids and alkaline pretreatments for 10g orange peel at 96 hr.

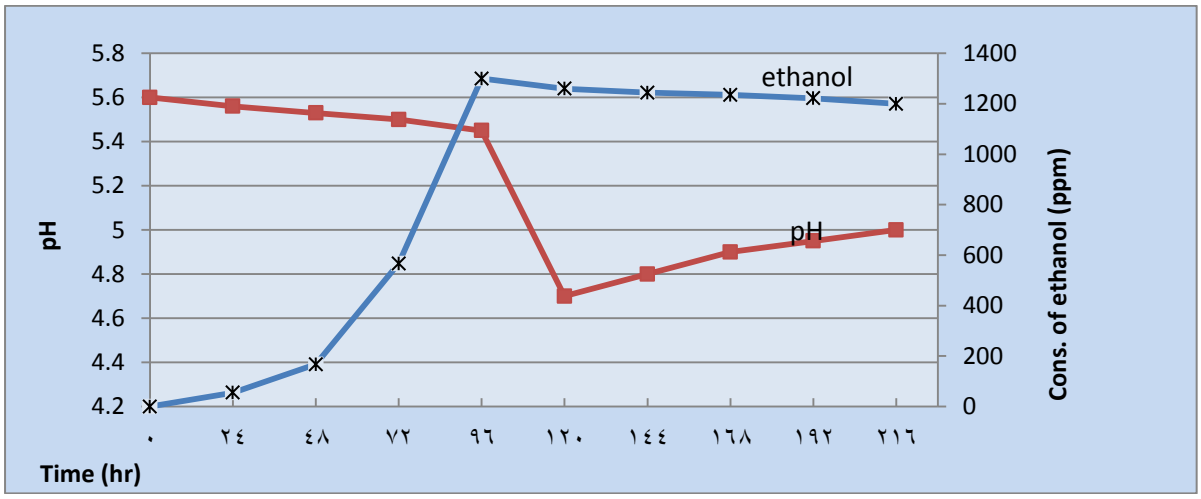

Figure 6. pH profile within the fermentation process with time.

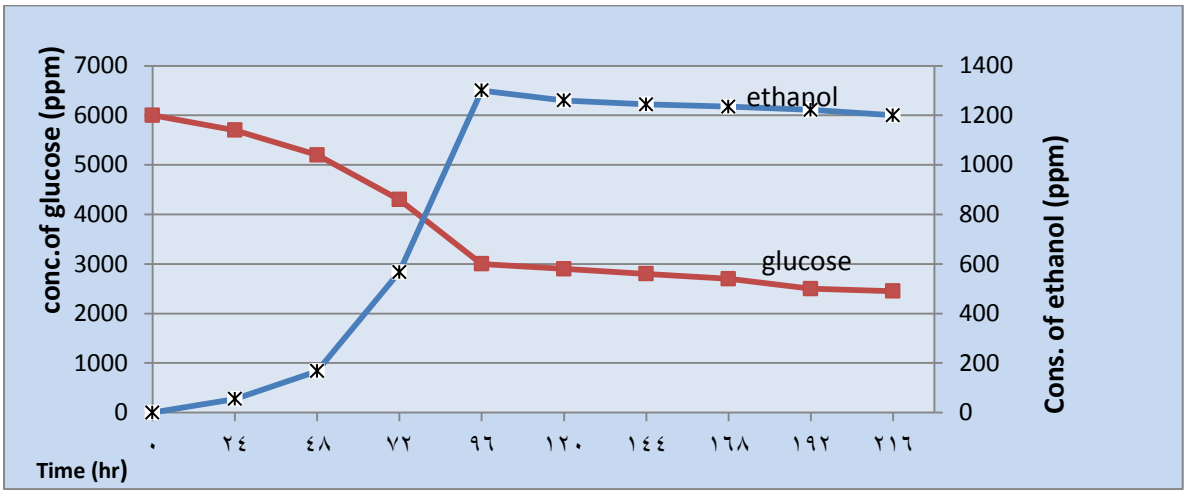

Figure 7. Ethanol and sugar concentration with time.

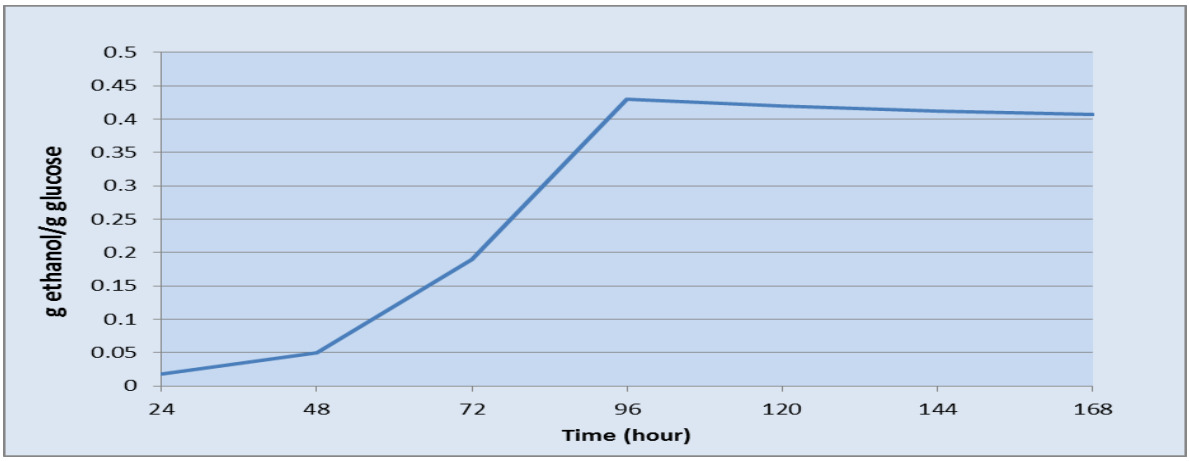

Figure 8. Ethanol yield with time