Production of Bioethanol from Olive Solid Waste "JEFT"

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Abstract

Olive solid residue (JEFT) is the solid waste generated during olive oil production process in three-phase olive mills. It consists of the remaining pulp of olive processing after the extraction of oil, as well as the cracked seeds of the olive fruits. As a lignocellulosic material, the hemicellulose, cellulose and lignin are the main components of olive stone. The present study standardized production of ethanol from olive solid wastes using *Saccharomyces cerevisiae* isolates. *S. cerevisiae* isolated from yogurt, grape and sugarcane. These isolates were identified according to morphological and biochemical characterization tests. The alcohol tolerance test indicated that *S. cerevisiae* tolerated up to 10% of ethanol in the medium. Optimization of culture conditions such as pH and temperature of yeast isolates was did. 10g JEFT subjected to hydrolysis to sugar by different concentrations of diluted sulfuric and hydrochloric acids. Among acid treatment, the maximum amount of reducing sugar was 312 mg/dl obtained by 5% HCl. Using combined microwave-acid treatment with 5% HCl followed by incubation at 90°C in shaker water path for 3 hours resulted in higher amount of reducing sugars reached up to 389 mg/dl. pH 4.5 and 30°C temperature for 72 hours were the optimum fermentation conditions. The maximum observed amount of ethanol production by using JEFT hydrolysate was (9.3 g/l). When compared with hydrolysis with conventional heating by dilute 5% HCl, the microwave assisted 5% HCl process improved the yield of ethanol by 33.3%.

1. Introduction:

Worldwide high demands for energy, uncertainty of petroleum resources, and concern about global climatic changes led to the resurgence in the development of alternative fuels. Ethanol considered a better choice as it reduces the dependence on crude oil and promises cleaner combustion leading to a healthier environment. Developing ethanol as fuel beyond its current role would require a cheap feedstock such lignocellulosic materials renewable nature, abundance, and low cost (Saha et al., 2005). Currently, ethanol produced from sugars, starches and cellulosic materials (Taherzadeh and Karimi, 2007).

The major producers of ethanol are Brazil and the US, which account for about 62% of world production. The major feedstock for ethanol in Brazil is sugar cane, while corn grain is the main feedstock for ethanol in the US (Kim and Dale, 2004).

Olive oil production is an important industry in many countries including Palestine. Olives and their oil have major contributions in the Palestinian economy. The annual production of olive fruits in Palestine is about 100,000 tons producing more than 40,000 tons of solid waste and about 25,000 tons of olive oil (Abu-Zreig & Al-Widyan, 2002; Albarran et al., 2006).

Lignocellulose represents as a building block of plant cell wall structure (Perez et al., 2002). Lignocellulosic materials such as agricultural and forest residues, crops and herbaceous materials in large quantities are available in many countries with various climatic conditions, making them suitable and potentially cheap feedstock's for sustainable production of fuel ethanol. Lignocelluloses are complex mixtures of carbohydrate polymers, namely cellulose, hemicellulose, lignin, and a small amount of compounds known as extractives.

Cellulose is the main structural polymer in plant cell walls and is found in a very organized fibrous structure. This linear polymer consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic bond. Due to this linkage, cellobiose is established as the repeat unit for cellulose chains.

Hemicelluloses consist of different monosaccharide units such as pentoses (xylose, rhamnose and arabinose), hexoses (glucose, mannose and galactose) and unonic acid (e.g. 4-o-methyl-glucuronic, D-glucuronic and D-galactouronic acids). In contrast to cellulose, hemicellulose an easy hydrolyzable polymer due to its branched nature and does not forming aggregates even when they are co-crystallized with cellulose chains.

In contrast to cellulose and hemicellulose, structure of Lignin is a complex, cross-linked polymer of phenolic compound monomers that form a large molecule structure. It is present in the cellular cell wall, conferring structural support, impermeability and resistance against microbial attack and oxidative stress (Perez et al., 2002).

The aim of this study was the production of ethanol from olive solid waste "JEFT" by different *Saccharomyces cerevisiae* isolates after pretreatment by physical and chemical procedures.

Gaza strip is one of the most crowded areas in the world. It suffers from lack of natural resources. In addition to the control of Israeli occupation on petrol and chemical supplies. This situation attracted our attention to look for alternatives such as biofuels. The importance of this study lies in twofold:

First, attempt to exploit the JEFT in the production of materials of economic importance such as ethanol. Ethanol considered as important material and has many applications as a solvent, disinfectant, or chemical feedstock. Ethanol used as a blending agent with gasoline to increase octane and reduce carbon monoxide and other smog-causing emissions.

Second, environmental importance as production process of olive oil, one of the main agricultural products in the Mediterranean area, leads to the generation of large quantities of liquid and solid wastes. As shown from previous studies, these wastes can represent an environmental hazard when disposed directly to the environment, due to their high organic load and toxic effect to microorganisms, plants and, as recently shown, to marine organisms (Azbar et al., 2004; Danellakis et al., 2011).

2. Materials and Methods:

2.1 Olive plant:

Olive tree (*Olea europae L.*) orchards are dominant crops in Mediterranean countries. Olives are one of main crops in terms of cultivation surfaces across the Mediterranean basin (Niaounakis & Halvadakis, 2004). The Mediterranean area alone provides 98% of the total surface area for olive tree culture and total productive trees, and 97% of the total olive production. Olive trees are grown everywhere in Palestine, but the greatest productive areas are located in the western and northern West Bank: About 90- 95 percent of the Palestinian olive harvest is used to produce olive oil: In past decade average oil production in good years has been around 20,000-25,000 tons. The quantity of olive oil produced in 2010 reached 23,754 tons (PCBS, 2011).

2.2 Microorganisms:

Microorganisms play a significant role in production of ethanol from renewable resources and thus, selection of suitable strain is essential for the individual process. Ethanol production is much more challenging and difficult when lignocellulosic and/or cellulosic materials are used as raw materials (van Zyl et al., 2007). Baker's yeast, *S. cerevisiae*, is widely used in ethanol production due to its high ethanol yield and

productivity, no oxygen requirement, and high ethanol tolerance (Olsson & Hahn-Hägerdal, 1993).

2.3 Methods:

The overall process involves the following steps- Pretreatment of the olive solid waste, Hydrolysis of the pretreated material to release reducing sugars and Fermentation of the released sugars, to produce ethanol.

2.3.1 Sample collection and isolation:

The yeasts (*S. cerevisiae*) were isolated from different sources of grapes juice, sugarcane juice, and traditional yogurt. Each juice exposed to air for 24 h and by using techniques such as serial dilution and spread plate method using yeast peptone glucose agar medium (YPG). The samples plated on YPG and incubated at 30°C for 72 hours. After incubation, the colonies plated on YPG medium supplemented with 30 mg/mL chloramphenicol and incubated at the same conditions (Thais et al., 2006). Pure culture of the colonies were prepared on YPG agar media by keeping the plate at 30^{0}C for at least 3 days and maintained on YPG agar slants at 4°C. Yeast cultures were stored in 40% sterile glycerol at - 80°C. The isolates sub-cultured on YPG agar media, incubated at 30°C for 3 days and then used to inoculate pre-culture broths.

2.3.2 Inoculum preparation:

The isolated yeasts and the commercial strain inoculated in YPG broth and incubated at 30°C for 12 h with constant shaking at 110 rpm. After incubation, a suspension was prepared and adjusted to an optical density of 0.1 at 660 nm. An aliquot of 50 mL of specific broth media used for carbon source assimilation, temperature tolerance test, and ethanol tolerance test. The volume of suspension (10 ml of inoculum in 40 ml of specific broth media) used that able to provide an absorbance between 0.1 and 0. 2 at OD 660 nm (Thais et al., 2006).

2.3.3 Characterization of the selected yeast isolates:

Yeast isolates identification based on the morphological characters (Kreger-Van Rij, 1984; Mesa et al., 1999) and physiological characters.

A. Morphological characterization:

Yeast isolates cultured by inoculation of 10ml of pre-activated culture in 100 mL of sterile YPG media broth in 250 mL conical flask, incubated at 30°C for 48 h. The grown yeasts examined for vegetative cells shape and budding pattern under phase contrast microscope (Olympus, Japan).

B. Physiological characterization:

The isolated strains along with reference *S. cerevisiae* (control) screened for their growth on different carbon sources as described by Wickerham & Burton (1948). Tolerance to ethanol performed by using the standard protocols (Bowman & Ahearan, 1975).

C. Carbohydrate source assimilation test:

Carbohydrate assimilation test performed by using different carbohydrates (Glucose, Galactose, Maltose, Xylose, Sucrose and Lactose) as carbon source. In this test, 50 ml of YP media, each containing a specific carbohydrate inoculated with yeast isolate. The inoculum incubated at 30°C for 96 hours with constant shaking at 150 rpm. Samples taken approximately after 24, 48, 72 and 96 hours. Yeast growth was measured in these samples as turbidity by using of a spectrophotometer (Chrom Teck, UV-1601, Taiwan) at a wavelength of 660 nm.

D. Tolerance to ethanol:

Tolerance of yeast stains to ethanol tested in comparison with the commercial strain *S. cerevisiae* as standard reference. 10 ml of 24 h old culture inoculated in 100 ml YPG broth and subjected to different concentrations of ethanol (5%, 8%, 10%, 13% and 15% ml/L) in the YPG broth. The inoculum incubated at 30°C for 4 days with constant shaking at 150 rpm. The growth curves constructed to find out the best growth at specific ethanol concentration (Khaing et al., 2008).

2.3.4 Optimization of the environmental condition:

A. Optimization of pH:

The isolates of yeast tested to determine the optimum pH for fermentation and for bioethanol production. The isolated yeasts and the commercial strain inoculated in 50 ml of liquid YPG media broth with different pH (4, 4.5, 5 and 5.5) at 30°C for 72 hours with constant shaking at 150 rpm. A pH meter used to measure the pH of each solution during and after establishment (Tahir et al., 2010).

B. Optimization of temperature:

The isolates of yeast tested to determine the optimum temperature for fermentation and bioethanol production. The isolates of yeast inoculated in 50 ml of liquid YPG media broth. The inoculums incubated in

flasks with different temperatures (25°C, 30°C, 35°C and 40°C) for 72 h with constant shaking at 150 rpm. Samples taken approximately after 2, 4, 6 and 24 hours (Neelakandan & Usharani, 2009).

2.3.5 Collection and preparation of olive solid waste (JEFT):

Fresh JEFT obtained from an olive factory in Rafah city in Gaza-strip in October 2012. For pretreatment purpose, samples soaked in water overnight to remove dirt and excess oil. The oil slowly left the solid waste to float on the water surface. Then the solid waste separated from the dirty water using a sieve and dried in air to make the weight constant before pretreatment. The biomass dried at 50°C by using oven until stabilization of weight (1day). The dried biomass subjected to mechanical treatment using stainless steel grinder mill. Stones with diameter above 1mm removed by using sieve and the cleaned JEFT stored at -20°C until use. Before using, the cleaned JEFT dried at 50°C for 1 day in oven (Mishra et al., 2011).

2.3.6 Hydrolysis process:

Hydrolysis process performed by acids and microwave to catalyze conversion of complex polysaccharides in the JEFT into simple sugars. The pretreated samples of the JEFT subjected to hydrolysis by diluted H_2SO_4 or diluted HCl. A round 10 g of powder JEFT placed in 250 ml rounded bottom flask fitted with a condenser and with a magnet stirrer bar for mixing. About 100 ml of diluted H_2SO_4 or 100 mL of diluted HCl added to the contents. Hydrolysis performed at various concentration of H_2SO_4 (3%, 5%, and 7%). This process proceeded at 90°C temperature in shaker water path for various periods of time (1, 2, 3, 5, and 7 hours). Then the reaction mixture neutralized with 4M NaOH solution (Shalabia, 2011).

2.3.7 Microwave-acid Treatment:

Microwave treatment carried out using a domestic microwave instrument (JAC, China). The microwave instrument operated at 2450 MHz and 450 W. For acid pretreatment, 100 ml of 5% HCl mixed with 10 g of JEFT in 250 ml Erlenmeyer flask for 5 min, and shaking well. The mixture then exposed to microwave for 5 min. The resulted mixture passed to shaking water path at 90°C temperature for 3 h. The reaction mixture neutralized with 4M NaOH solution (Binod et al., 2012).

2.3.8 Estimation of reducing sugars:

The amount of reducing sugars was estimated by using glucose assay kit (Diasys, Germany).

2.3.9 Estimation of ethanol:

The ethanol was estimated calorimetrically by using ethanol assay kit (Biovision, USA).

3. Results and Discussion:

3.1 Characterization and identification of the isolated *S. cerevisiae* strains:

3.1.1 Morphological characterization:

Morphological characterization is an important tool for classic identification of yeasts. We observed complete colonies of 2–3 mm in diameter, slightly convex, of a smooth, creamy consistency, white to cream in color and having a sweet smell that is typical of yeast (Figure 1). Similar results reported by Tikka et al. (2013). Yeast strains produced different types of colonies on YEPDA medium such as raised, creamy white color colonies. Microphotographs of different colonies from different sample. Strains were observed for Saccharomyces characteristic oval cell shape and budding characters. Out of fifteen isolates, seven isolates showed oval cell shape with budding character (Figure 1).

Figure 1 *S. cerevisiae on yeast extract peptone glucose agar plate and the microscopic morphology of S. cerevisiae*

3.1.2 Biochemical characterization:

A. Carbohydrate source assimilation test:

Yeast isolated from sugarcane, grape and yogurt was able to utilize various sugars such as glucose, maltose, sucrose, galactose but not lactose and xylose. The isolates compared to the commercial reference of *S. cerevisiae*. Similar results reported by Kumar et al, (2011). They tested by the fermentation patterns of carbon sugars, some of the sugars like glucose, galactose, maltose, sucrose, raffinose were positive in fermentation process which indicated by color change from red to yellow due to acid production (Table 1).

Maltose taken up via maltose permease and then hydrolyzed intracellular by maltase into two units of glucose. Maltose permease encoded by MALT. MAL genes cannot induced if maltose cannot transported into the cell (Goffeau, 2000).

Table 1 *Carbohydrate assimilation for the reference S. cerevisiae (control) and the different isolates of S. cerevisiae*

| Lactose	Sucrose	Xylose	Maltose	Galactose	Glucose	
-	+	-	+	+	+	*S. cerevisiae* (yogurt)
-	+	-	+	+	+	*S. cerevisiae* (grape)
-	+	-	+	+	+	*S. cerevisiae* (sugarcane)
-	+	-	+	+	+	*S. cerevisiae* (control 1)
-	+	-	+	+	+	*S. cerevisiae* (control 2)

B. Ethanol tolerance test:

The growth of yeast isolates in different ethanol concentrations given in Figures 2-5. Yeast isolated from sugarcane showed that it can tolerate ethanol at 10% concentration and gradually decreased at higher concentrations. Similar results reported by Tikka et al. (2013), they screened seven strains of *S. cerevisiae* obtained from different fruit sources for ethanol tolerance. The results obtained in this study showed a range of tolerance levels between 7%-12% in all isolates. Ethanol well known to inhibit yeast growth and viability, affecting various transport systems, such as- the general amino acid permease and glucose uptake process (Arroyo-Lopez et al., 2010). It can cause damage to mitochondrial DNA and degrades biological membranes in yeast cells and inactivates some enzymes, such as hexokinase and dehydrogenase (Lopes and Mauro, 2001; Mobini-Dehkordi et al., 2007).
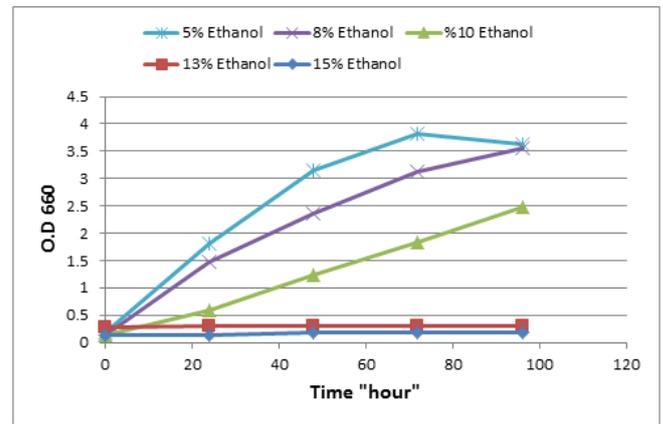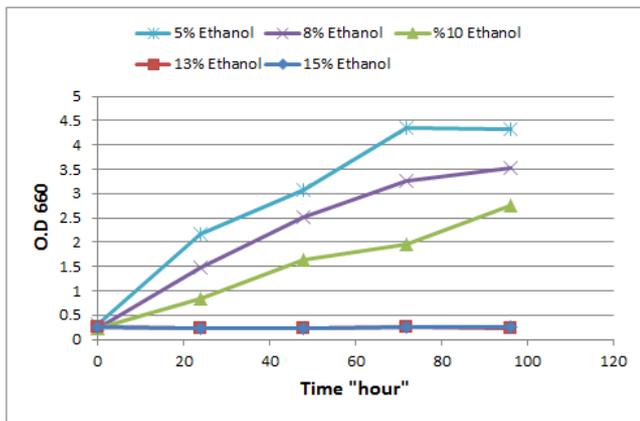

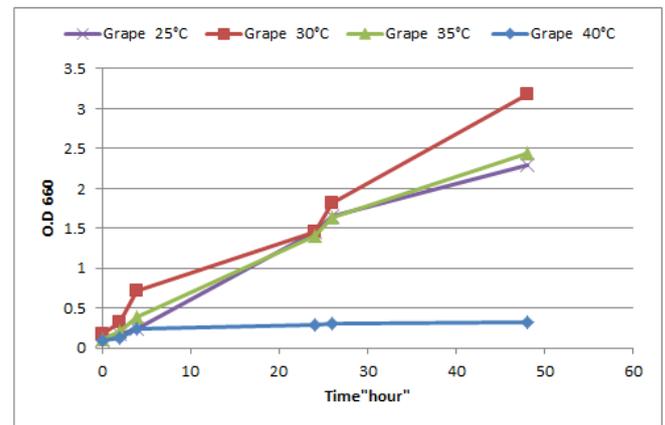**Figure 2** *Growth of S. cerevisiae isolated from sugarcane at different ethanol concentration*
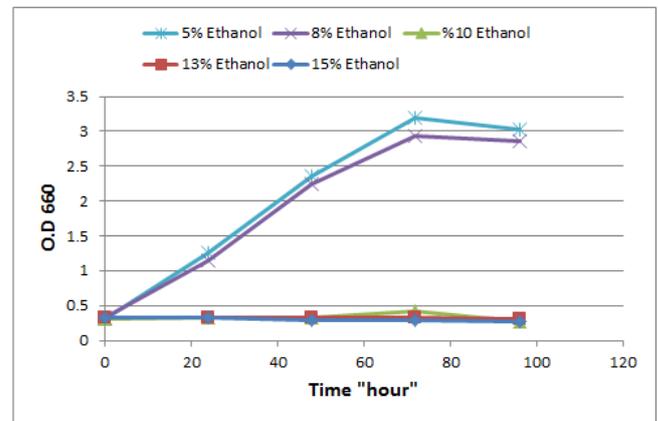
Figure 3 *Growth of S. cerevisiae isolated from grape at different ethanol concentration*
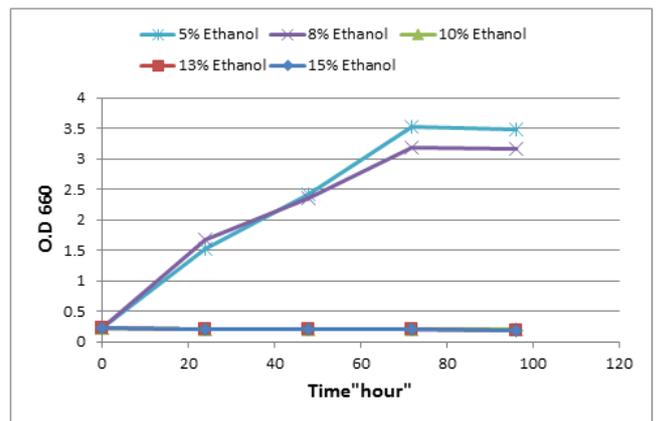
Figure 4 *Growth of S. cerevisiae control 1 atdifferent Ethanol concentration*

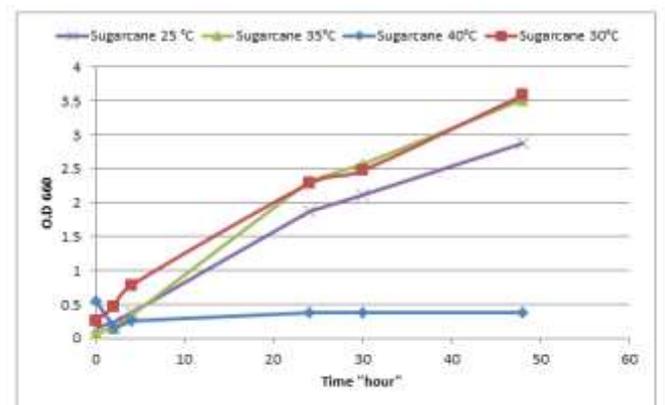**Figure 5** *Growth of S. cerevisiae isolated from yogurt at different ethanol concentration*

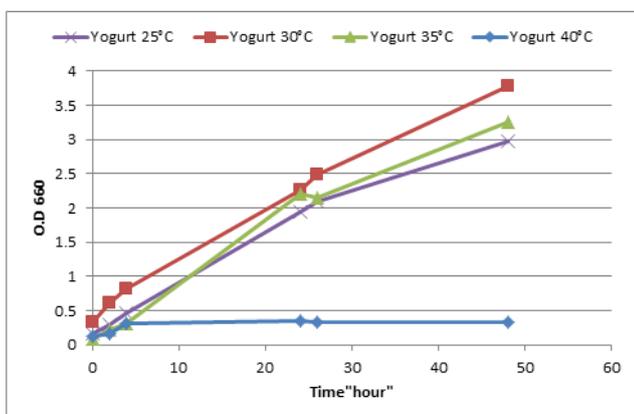### 3.1.3 Optimization of growth conditions:

A. Optimization of Temperature:

Temperature has a marked influence on the production of ethanol. According to Rivera et al., (2006) suitable temperature in fermentation process is the good condition for the yeast to react. Too high temperature kills yeast, and low temperature slows down yeast activity. Thus, it is required to keep a specific range of temperature. Normally, ethanol fermentation conducted at temperature ranges between 30-35°C where ethanol produced at highest concentration as stated by Shuler and Kargi, (2002).

The isolated *S. cerevisiae* and control strains tested for their growth at different temperature. The optimum temperatures for most of the *S. cerevisiae* strains estimated in the temperature range from 30°C to 35°C. As shown in Figures 6–9, the optimum growth temperature for all isolates of *S. cerevisiae* found at 30°C.

Figure 6 *Growth of S. cerevisiae isolated from yogurt at different temperatures*

Figure 7 *Growth of S. cerevisiae isolated from grape at different temperatures*

Figure 8 *Growth of S. cerevisiae isolated from sugarcane at different temperatures*
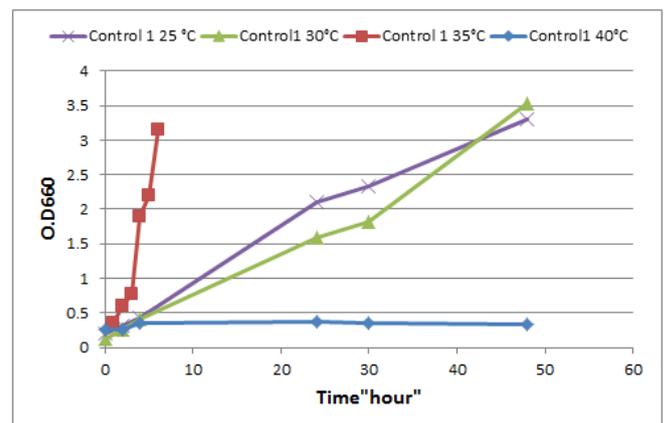
Figure 9 *Growth of S. cerevisiae control 1 at different temperatures*

B. Optimization of pH:

Hydrogen ion concentration has a significant influence on industrial fermentation due as much to its importance in controlling bacterial contamination as its

effect on yeast growth, fermentation rates and by-product formation (Wayman & Parekh, 1990). The growth of *S. cerevisiae* isolates at different pH given in Figures 10–13. The isolated yeasts from yogurt and grape recorded maximum population at pH 4.5 while the yeast isolated from sugarcane recorded maximum population at pH 4. Above pH 5.0 yeast populations declined, the optimal pH for ethanol production was around 4.5. Similar results reported by Lin et al., (2012).

Increased substrate supply did not improve the specific ethanol production rate when the pH value was not controlled and pH 4.0-5.0 was the optimal range for the ethanol production process. The most likely explanation for the observed reduction in ethanol production when the extracellular pH deviates too much from the optimal range, it may become too difficult for the cell to maintain constant intracellular pH, and the enzymes may not function normally. If the enzymes are deactivated, the yeast cell will not be able to grow and make ethanol efficiently (Narendranath & Power, 2005).
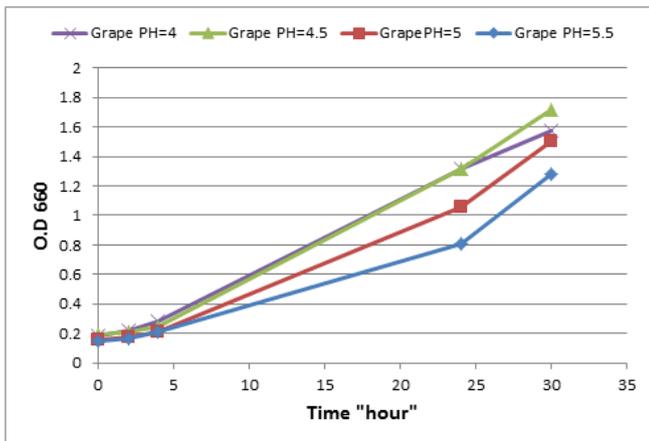

Figure 10 *Growth of S. cerevisiae isolated from grape at different pH*
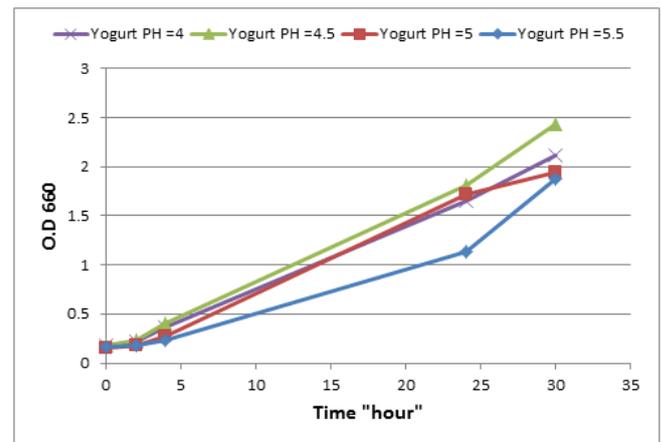

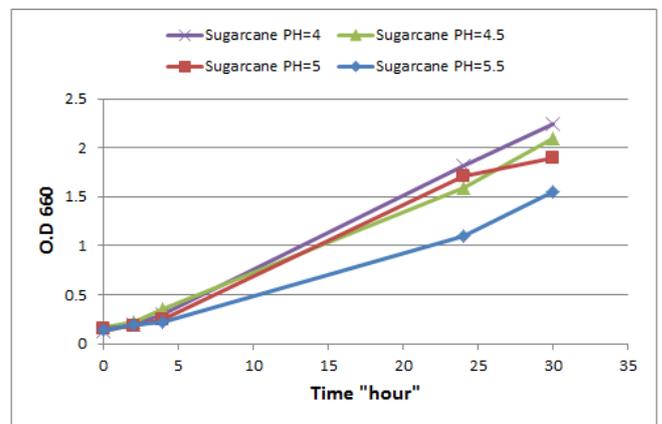**Figure 11** *Growth of S. cerevisiae isolated from yogurt at different pH*

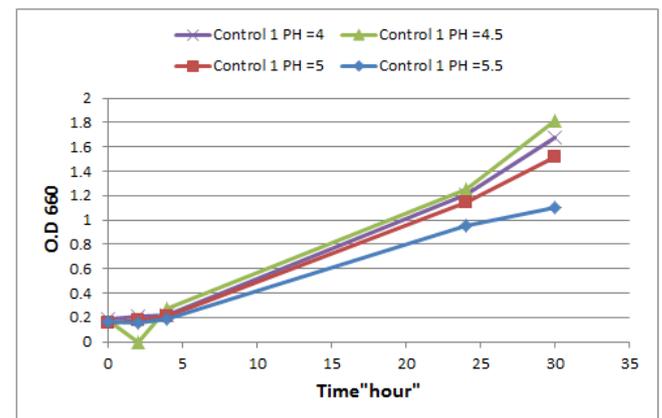**Figure 12** *Growth of S. cerevisiae isolated from sugarcane at different pH*

Figure 13 *Growth of S. cerevisiae control 1 at different pH*

3.2 Pretreatment of olive solid waste:

3.2.1 Hydrolysis process:

A. Effect of different concentrations of HCl:

Effect of different concentrations of HCl on hydrolysis of olive solid waste tested with different time intervals of 1 hour to 7 hours by estimating the concentration of reducing sugar (Table 2). At 3 hour of hydrolysis process, the maximum amount of reducing sugar [312 mg/dl] observed at 5% HCl concentration (Table 2).

B. Effect of different concentrations of H_2SO_4:

Effect of different concentrations of H_2SO_4 on hydrolysis of olive solid waste tested at different time intervals of 1 hour to 7 hours by estimating the concentration of reducing sugar percentage (Table 2). At 3 hour of hydrolysis process, the maximum concentration of reducing sugar was 274 mg/dl in 7% H_2SO_4 concentration (Table 2).

3.2.2 Microwave assisted with 5% HCl pretreatments:

The results showed that the pretreated substrate with microwave assisted 5% HCl at 3 hours had significantly produce higher concentration of reducing sugar yield [389 mg/dl] than the samples treated with Chemical pretreatment alone 5% HCl (Table 2).

A review of published studies agree with our results which indicated that microwave pretreatment in the presence of chemical reagents would be more effective. Further, Sree (1999) stated that efficient and rapid heating by microwave radiation able to accelerate chemical reactions. Volumetric and selective heating of lignocelluloses by microwave, facilitates the disruption of their recalcitrant structures more efficiently (Hendriks & Zeeman, 2009).

Table 2 *Effect of different concentrations of HCl, H_2SO_4 and microwave assistant 5% HCl on hydrolysis of olive solid waste at different time intervals (mg/dl)*

	HCL 3 %	HCL 5 %	HCL 7 %	H_2SO_4 3 %	H_2SO_4 5 %	H_2SO_4 7 %	Conc. HCL	Conc. H_2SO_4	Microwave With 5% HCL
1 Hour	117.4	168	145.4	149.4	93.2	110.6	43.2	23	311
2 Hours	173.6	145.6	214	153.6	162.4	167.4	63.8	25	384
3 Hours	169.4	312	288	155	246	274	67.2	41	389
5 Hours	138.2	192	224	126	170	221	70	55	379
7 Hours	125.2	171	220	113.2	151	220	65	51	351

3.3 Fermentation process:

During fermentation process, various parameters such as pH, reducing sugar percentage and temperature assayed (Table 3). The final pH of the olive solid waste was about 4.5, and reduction in the reducing sugar content as recorded as it shown in the Table where the fermentation process slowed down after the first day.

Table 3 *Concentration of glucose during fermentation process by the different yeast isolates by using at 10% inoculums size, pH 4.5 at 30°C for 5 days, after using microwave assisted 5% HCl treatment on hydrolysis of olive solid waste for 3 hours mg/dl*

Microwave with 5% HCL	Yogurt 1	Yogurt 1.1	Grape 2	Grape 2.1	Sugarcane 3	Sugarcane 3.1	Control 1	Control 1.1	Control 2	Control 2.1
0 Time	1314									
1 Day	5.5	6	6.1	5.7	17.2	17.8	7.2	6.5	28.5	29.6
2 Day	5.1	6	6.1	5.7	8.9	10.3	5.9	5.6	26.9	29.3
3 Days	4.8	5.6	5.9	5.3	6	6.3	5.6	5.4	10.4	11.9
5 Days	4.5	5.2	5.8	5.8	5.4	5.3	5.3	5.4	5.6	9.9

During fermentation, process various parameters such as pH, reducing sugar concentration and temperature were assayed (Table 4). The final pH of the olive solid waste was about 4.5, and reduction in the reducing sugar content was recorded at zero time was 1016 mg/dl. Through the Table 4 note that the fermentation process occurred on the first day and then stopped.

Table 4 *Concentration of glucose during fermentation process by the different yeast isolates at 10% inoculums size, pH 4.5*

at 35°C for 5days. After using 5% HCl pretreatments on hydrolysis of olive solid waste for 3 hours (mg/dl)

5%HCL	Yogurt 1	Yogurt 1.1	Grape 2	Grape 2.1	Sugarcane 3	Sugarcane 3.1	Control 1	Control 1.1	Control 2	Control 2.1
0 Time	1016									
1 Day	5.9	5.8	6.8	6.3	13.4	11.7	11.7	6.8	28.7	29.4
2 Day	5.7	5.7	6	6.2	7.5	8.9	10.1	6.4	16.5	15.9
3 Days	5.6	5.7	5.7	5.5	6.1	6.3	6.6	6.1	7.3	6.2
5 Days	5.4	5.5	5.7	4.3	6.1	6.3	6.4	6	5.3	5.7

3.4 Ethanol yield:

The results for ethanol production by yeast fermentation using three isolates of *S. cerevisiae* and two control strains shown in Table 5. The concentration of ethanol in the reactor that contained pretreated (using microwave assistant 5% HCl pretreatments) olive solid waste was much higher than in the pretreated one with 5% HCl. The amount of ethanol detected in those cultures reached up to 9.3 g/L after 3 days of yeast fermentation by using yogurt *S. cerevisiae* isolate.

Table 5 *Estimation of ethanol yield from pretreated olive solid waste by two methods (5% HCl, and microwave assisted 5% HCl) g/L*

Control 2.1 g/l	Control 2 g/l	Control 1.1 g/l	Control 1 g/l	Sugarcane 3.1 g/l	Sugarcane 3 g/l	Grape 2.1 g/l	Grape 2 g/l	Yogurt 1.1 g/l	Yogurt 1 g/l	
6.2	6.3	5.9	6.2	7.5	7.3	6.5	6.4	8.4	8.2	**5% HCl**
7	6.8	6.4	6.6	7.4	7.6	6.3	6.9	9.3	8.6	**Microwave with 5%HCl**

4. Recommendations:

The present work is part of extensive research devoted to meeting challenges and requirements that human society currently encounters in sustainable development. Producing fuel from renewable resources is becoming an important issue for the whole world. Therefore, the work needs to continue for further development of ethanol production from biomass. The following suggestions could be of interest for future studies:

1. It is recommended further to evaluate cellulose from olive solid waste as the substrate for industrial bioethanol production in addition to other common cellulosic biomass resources like market residue, wood, municipal solid wastes, wastes paper, agricultural residues and industrial residues.
2. Better assimilation of C5 and C6 sugars by using genetically engineered microorganisms recommended.
3. Alternate processing methods to get higher release of reducing sugars must tried to make the technology more efficient and profitable are required. Future work have to focus on the scale-up of microwave-based pretreatment of lignocellulosic biomass.

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إنتاج الإيثانول الحيوي من نفايات الزيتون الصلبة "الجفت"

كلمات مفتاحية:
نفايات الزيتون الصلبة،
اللجنين سيليولوز،
المعالجة المسبقة،
التحلل المائي الحمضي،
الإيثانول الحيوي.

عملت الدراسة الحالية على تصميم ظروف قياسية لإنتاج الكحول من حلامة المخلفات الصلبة للزيتون باستخدام عزلات من خميرة الساكاروميس سيرفيسيا. أظهر اختبار تحمل الكحول الإيثلي قدرة بعض العزلات على تحمل حتى 10% من الكحول الإيثيلي في وسط النمو. تم معايرة ظروف النمو والتخمر المثلى لجميع العزلات شاملة درجة الأس الهيدروجيني ودرجة الحرارة. تم تعريض 10 جرام من الجفت للتحلل المائي إلى سكر الجلوكوز باستخدام تراكيز مختلفة من حامض الكبريتيك وحامض الهيدروكلوريك. تم الحصول على أعلى كمية من السكر المختزل وهي 312 مغ/100مل عند استخدام 5% من حامض الهيدروكلوريك. عند مزاوجة معالجة 5% حامض الهيدروكلوريك مع معالجة الميكروويف متبوعا بحضانة الخليط على حرارة 90 درجة مئوية لمدة ثلاث ساعات في حمام مائي هزاز تم الحصول على كمية من السكر المختزل تساوي 389 مغ/100 مل. قد وجد أن درجة الحموضة 4.5 وحرارة 30 درجة مئوية مثلت الظروف المثلى لعملية التخمر لفترة 72 ساعة. قد لوحظ بأن أعلى كمية من الإيثانول تم الحصول عليها عند استخدام مهضوم الجفت تساوي 9.3 جرام لكل لتر من المحلول المستخدم. عند مقارنة نتائج التحلل المائي باستعمال 5% من حامض الهيدروكلوريك مصحوبا بالتسخين التقليدي مع مزاوجة الحامض مع التسخين بالميكروويف وجد أن استخدام الميكروويف قد حسن محصول الإيثانول الناتج بمقدار 33%.