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Bioethanol (Biofuel) Production from Low Grade Dates

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Abstract

Bioethanol production from sugar fermentation is one of the most sustainable alternatives to substitute fossil fuel. production of bioethanol from low grade dates which are rich of sugars. An available sugar from a second grade dates (reduction sugar) was 90g/l in this study. Sugar can be served as essential carbon sources for yeast growth in aerobic condition and can also be converted to bioethanol in anaerobic condition. The effect of various parameters on bioethanol production, fermentation time, pH-values, inoculum size and initial sugar concentration were varied in order to determine the optimal of bioethanol production**.** The highest bioethanol yield was 33g/l which was obtained with sugar concentration 90 g/l, inoculum size 1%, 52h time and pH-value 5.

Keywords: Bioethanol, Fermentation, Saccharomycie cerevisiae, Low grade Iraqi dates

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1- Introduction

The planet is sink in energy problems due to development in different life sectors that lead to intensive consumption of the fossil fuels also the high prices of fossil fuels in all over the world. The world depends on nonrenewable energy sources for transport, heat and/or power generation. Fossil fuels are currently the main energy source, providing an estimated 78.4% of the global final energy consumption.

Increased awareness of environmental problems like greenhouse gases and global warming seeks for alternative energy sources that are competitive to be more sustainable and economical [1]. Biofuel is one of the sustainable fuel that can be produced from biodegradable part of products like cellulosic biomass, waste of chicken feathers, food and organic waste such as agricultural waste by carbon fixation biologically [2].

Some other sources of biofuel production are waste cooking oil and plants that generate carbohydrates or fats which capable to be a source of biofuel [3]. Algae is one of the most favorable sources of protein supplements, biofuel, and organic fertilizer owing to their advantages like capacity to use fresh, marine or wastewater, reduce greenhouse gas from the environment, non-competing with food yields, and non-requirement of fertile land Low cost and large scale process for biofuel production from municipal and agricultural waste which are more afforded from research seekers [4]. In Iraq, the best standard raw material for bioethanol production is Date palm for two reasons; firstly the cost of production dates palm juices is usually less expensive than other sources, secondly juices of date palm fruits are purer than the other sources [5].

Microorganisms like Saccharomyces cerevisiae play an important role in one of the safest and most successful biological reactions in which biological reaction return the balance within the environmental system, in addition to converting the waste of date palm fruit to economic profit products [6].

Fermentation is biological process in which sugars are converted to alcohol in an aerobic condition by the action of microorganisms [7]. Yeast (Saccharomycie cerevisiae) and Zymomonas mobilis of bacteria are microorganisms capable of producing ethanol as a product by converting sugars into ethanol. The most common microorganism to produce bioethanol is *Saccharomyces cerevisiae*, because it is already safer to produce and had accepted as non-pathogenic, this organism can be easily grown on simple and cheap media compared to cell cultivation to other microorganisms [8]. Parameters had a huge effect on yeast cells broth such as pH, amount of oxygen, the amount and type of nutrients in broth, and temperature. 30 and 35°C was an optimum temperature for Saccharomyces cerevisiae [9].

As the fermentation is anaerobic condition, no oxygen was supplied. By controlling the process conditions as well as possible for a specific process ethanol is the main product produced when Saccharomyces cerevisiae fermented sugars. The “Date Strategy Report” in Iraq specified that 35.7% of the date crop (the low grade dates) are used for animal as a food source to the sheep herders and dairies and from the total production 14.3% is wasted [10]. Low grade Iraqi date is commonly used as animal food was selected from marketplace. Carbon source, nitrogen source in addition to essential minerals and vitamins are nutrient requirements for the *Saccharomyces cerevisiae* to generate energy and cellular growth which present in the composition of date extracts.

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pH, time, inoculum size and initial sugar concentrations were factors that affected the mechanism of ethanol production during the fermentation procedure. Waste palm date fruit is recycled in this study to produce bioethanol by used saccharomyces cervisiae. Evaluation of the influence of different conditions, such as fermentation time, pH-values, inoculum size, and initial sugar concentration on fermentation performance in order to determine the optimal bioethanol production

2- Experimental Work

2.1. Substrate Preparation

Samples of date are weighted. Date flash separated from date pits because high sugar content that is used in the preparation process of substrate. Date flash is washed with distaled water twice and mixed with deionized water with ratio (2/5)(w/w sample to water)[11]. The extraction of sugar date is affected by the temperature of the water and time of extraction, as well as mixing of particle of the date flash. Mixture of flash date is blended by using home blender to form suspension at 25^{o}C without heating [12].

Fig. 1. The suspension of date flash after mixing in blender at 25^{o}C

After soaking for 24 hrs suspension of date flash was filtrated through a piece of cloth to separate dissolved and non-dissolved particles in order to form date juice. Date juice filtrated through filtered paper (what man cat no: 1441) to remove particles which were passed through the piece of cloth. Solid suspension and Fibers in date juice were removed by centrifugation at (8000 rpm) for 20 minutes in a centrifuge. The date juice passed through filter (mille pore 0.45mm) to remove impurities and prevent any microbial growth that effected on *saccharomyces cereivisiae* growth.

2.2. Mineral Salt Media Preparation (MSM)

This media equipped according to Rahna [13], the preparations started with dissolving different chemical compounds showed in Table **1** in one liter of deionized water at pH 5.0.

MSM was sterilized at 121˚C for 15 min by autoclave (Hirayama/ Japan). This media was used to activate *saccharomyces cerivisiae* to growth.

Table 1. Chemical compounds used for preparations MSM

Component	Weight (g/l)
$(NH_4)_2SO_4$	1
Yeast extract	1
K_2HPO_4	1
$MgSO_4.7H_2O$	0.2
$CaCl_2.H_2O$	0.1
$FeCl_3$	0.002
Glucose	2

Fig. 2. Mineral salt media in Magnetic stirrer and hot plat

2.3. Effect of Various Parameters on Ethanol Production

a. Effect Time on Production of Bio-ethanol

Time course was considered to be estimated for an optimum period time for ethanol production process to produce high concentration of ethanol and the substrate of date juice was prepared.

100 ml of the substrate for fermentation process was put in flask 300ml .After adding 1%(w/v) of yeast, the first one in aerobic condition for 24h incubator shaker 120 rpm at 30℃ at PH=5 the second step in anaerobic condition in the shaker 120 rpm at 30℃ for 28h.

b. Effect of pH on Production of Bio-ethanol

Effect of PH on bio-ethanol production was studied. Different substrates were prepared in different PH (4.5, 5, 5.5, 6, and 6.5) to study the effect of PH on biomass and bioethanol production by added few drops of HCl, 100 ml of substrate was prepared for fermentation process. Date juice filtrated by passing it through filter (mille pore 0.45mm) and added (1)%(w/v)of yeast. After 24h of aerobic conditions incubator shaker 120 rpm at 30℃ to help saccharomyces cerivisiae to growth in the media then 24h in anaerobic condition to produce bioethanol in shaker 120 rpm at 30℃.

c. Effect Inoculum Size on Bio-ethanol Production

Different concentrations of inoculums include (0.25, 0.5, 1, 2, and 3) % was tested to estimate the best inoculums concentration for ethanol production during fermentation process and biomass growth.

d. Effect of Initial Sugar Concentration on Bio-ethanol Production

Different concentration of sugar was investigated to study (0%, 25%, 50%, and 75%) from date juice. Substrate was prepared in flask 300ml then 1% of yeast added to substrate at PH=5. The flask aerated for 24 hrs and 30℃ then 24hrs. of anaerobic condition in shaker 120 rpm at 30℃ to produced bioethanol.

2.4. Estimation of Residual Sugar

The residual sugar in fermentation broth filtrate is estimated by using suitable glucose oxidase kit, (Croma test MR 4×250) by Linear Chemicals was used, date of production 2015. The calculations and preparations conducted to manufactures procedures, were :

1- Blank was prepared by adding 3 mL from reagent (R1) to number of remarked glasses tubes. The R1 reagent was mono reagent, consists of 100000 mol/L at pH 7.5 phosphate buffer, 10 KU/L < glucose oxidase 2 KU/L < peroxidase , 4-aminoantipyrine 500 mol/L, phenol 5000 mol/L .
2- Standard sample was prepared by adding 30 µL of glucose standard solution to 3mL reagent (R1). Standard contains, glucose 1000 mg/L (5550 mol/L).
3- 30 µL of each sample (supernatant) was added to 3ml of reagent (R1). Each tube was mixed well and incubated for 10 minutes in room temperature or 5 minutes in incubator with temperature 37°C. The absorbance of samples was measured at 500nm spectrophotometer, then the reducing sugar concentration was calculated by the following equation:

$$\text{Glucose concentration mg/dL} = \frac{\text{absorbance reading for sample}}{\text{absorbance reading for standard}} \times C \text{ standard} \quad (1)$$

2.5. Measurement of Biomass

To determine the biomass by drying weight at the end of incubation time, the substrate is centrifuged at 8000 rpm for 20 min. After weighting the filter paper then filtering the substrate and drying it in oven at 50 ℃ and reweighted. The difference of the initial and final weight of filter paper gave the amount of biomass and calculated by equation (2) :

$$\text{Biomass (g/l)} = \frac{initial\ weight - final\ weight}{volume\ of\ sample} \times 1000 \quad (2)$$

2.6. Determination of Bioethanol Concentration

Concentrations of bioethanol were determined by High Performance Liquid Chromatography (HPLC) according to the operating conditions below in the Table (2). The column used in analyzing was JIODS C18, USA (250×4.6 Id) with mobile phase 10% and 90% acetonitrile with flow rate 1mL/min. Column oven temperature at room temperature with UV (UV-visible spectrophotometer at 210 nm detector. JSR/ Korea). Operation condition are shown in the Table.2.

Table 2. HPLC operating condition

Condition	Range
Column temperature	80°C
Detector temperature	55°C
Flow rate	1.00ml/min
Injection volume	2µl
Mobile phase	mark water
RUN TIME	20MIN

3- Results and Discussion

3.1. The Effect of Time on Bio-ethanol Production

Shorter fermentation time causes insufficient growth of microorganisms that finally causes incompetent fermentation. Instead, longer fermentation time causes microbial growth toxicity effects due to the high ethanol concentration in the fermented broth [14]. The results of the fermentation in batch experiment are presented in the figure (3), which shows the kinetic profiles of the dates sugar and bioethanol resulting from the fermentation process, initial sugar concentration (46g/l) fermented by S.cerevisiae. After 28h of fermentation time (38g/l) consumption of sugar, the ethanol yields (30g/l). The optimum fermentation time for this production was (52h). This is conformity with the findings [14]. The sugar concentration decreased with time as showed in Fig. **3**. Biomass increased with time during (24h) with time biomass decreased because concentration of the fermentation medium began to decrease with time in this study this similar to other study [4].

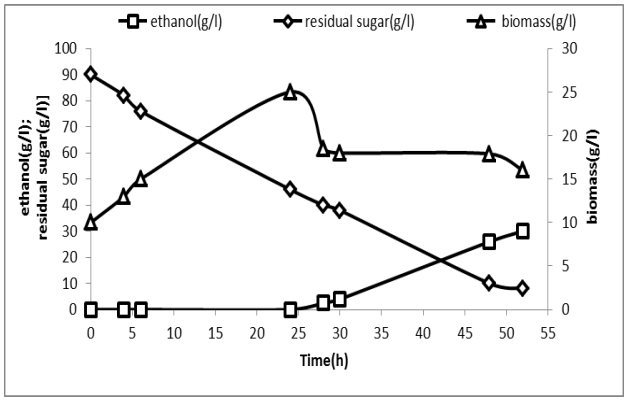

Fig. 3. Effect of time on bioethanol production at sugar concentration (90g/l), inoculum size (1%) and pH-value (5)

3.2. Effect of PH on Bioethanol Produuction

In general, pH-values in fermentation memberane affect the absorbency of some important nutrients into the cell. The best pH range for S.cerevisiae used in fermentation for bioethanol production is (5) [15,16].

The results obtained in figure(4) shown below the different bioethanol production with different pH-value (4.5,5,5.5,6,6.5). The bioethanol production increases with the increase in PH –values and reaches a maximum production, for a PH- values equal to 5 (33g/l). These results were similier to other studies like [20].The bioethanol production decreased slightly for the pH-values higher than 5. These results are comparable to those described by [17].

Fadel M. [18] was reported that high bioethanol production was obtained by using initial pH-values 5 to 6 , which was in agreement with the results of this study. Media of date juice was prepared in a way to get an initial total sugars of (90g/l), 24h of earobic condition to activation the sacchromycieses cerivisiae at 30°C for 120 rpm. Biomass growth and ethanol production and residual sugar were estimated to obtain the optimum values at pH 5 as shown in the figure (5).

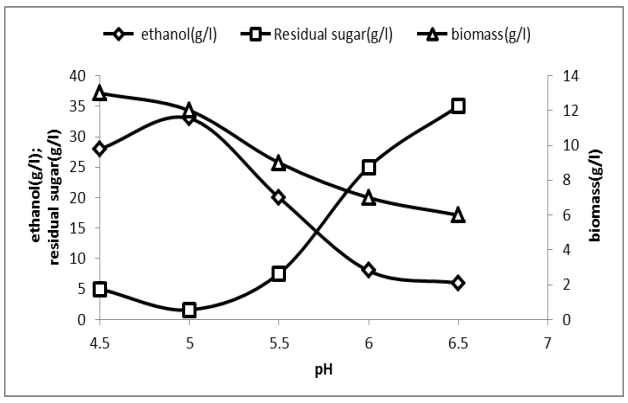

Fig. 4. Bioethanol results in different PH- value after (52hrs.) by using (1%) of inoculum size

The biomass increased during (24hrs.) then decreased with time similar to explination in [7] as shown in the Fig. **5**. Residual sugar concentration decreased with time due to converted it to bioethnol with time as showen below in the Fig. **6**.

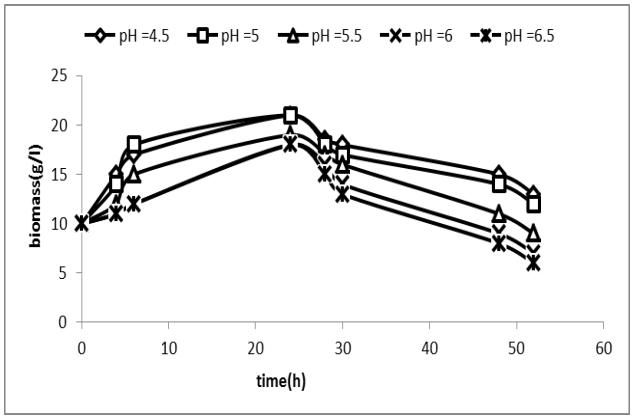

Fig. 5. Effect of pH on biomass at different PH-values during (52hrs.), initial sugar con.90g/l and inoculum size (1%)

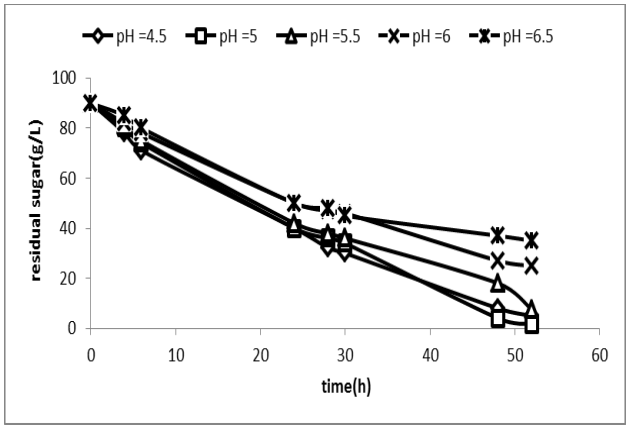

Fig. 6. Effect of pH-values on suar consumption during (52hrs.) at inial sugar con.(90g/l) andinoculum size (1%)

3.3. Effect of Inoculum Size on Bioethanol production

Five different inoculum size (0.5%,1%,2%,4%,6%) were tested to predicate the best additional of inoculum to determine the effect of inoculum size on kinetic parameters of ethanol fermentation from low grade dates.

The maximum bioethanol yield (30g/l) and maximum biomass (15g/l) were found with 1% inoculum size which product (30g/l) ethanol.

Fermentation process began and continued 52h at 30°C and 120rpm. Optimum ethanol concentration yield at inoculum 1% was(30g/l) other inoculum 0.5% ethanol yield (20g/l), 2% inoculum size ethanol yield(23g/l), 4% inoculum ethanol yield (15.3 g/l), and 6%inoculum ethanol yield (12.4g/l) as shown in Fig. **7**

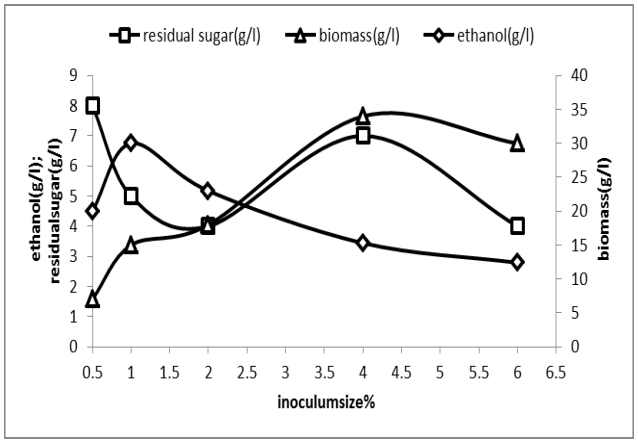

Fig. 7. Results of bioethanol, biomass and residual sugar after (52 hrs.) at different inoculum size, pH-value 5 and initial sugar con (90 g/l)

Ethanol yield decrease with increased inoculum size due to increase biomass which required more substrates in media with time sugar concentration decreased. The results in this study similar to other study [19] sugar conversion was continuing simultaneously during the fermentation, and after 52h of fermentation, sugar was completely consumed in both cases as shown in Fig. **8**

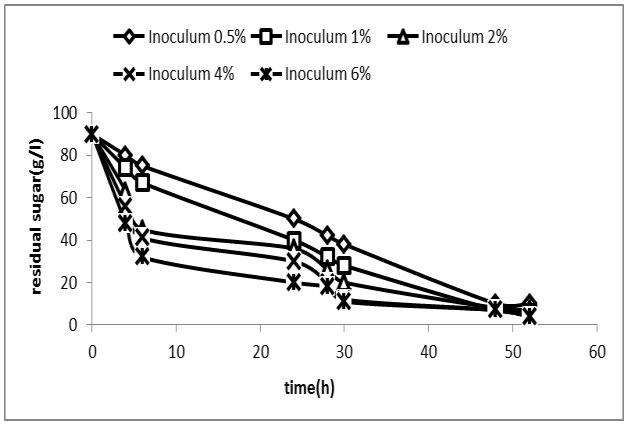

Fig. 8. Effect of inoculum size on sugar consumption during (52hrs.), initial sugar con.(90 g/l) and (1%) inoculum size

After consumption of sugar, both production of ethanol and growth of biomass slowed as shown in the Fig. **9**

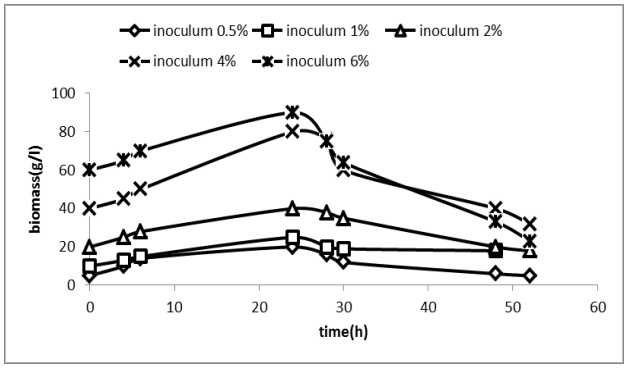

Fig. 9. Effect of inoculum size on biomass growth during (52hrs)

3.4. Effect Sugar Concentration on Bioethanol Production

Four different sugar concentrations is tested to predict the best sugar concentration in fermentation process. These experiments are prepared in four different concentration by dilution the date juice with distilled water according these ratio (0%,25%,50%,75%) to determine the effect of sugar concentration of ethanol fermentation from low grade dates figure (10) shows the ethanol production(g/l), Fermentation process began and continued 52 h at 30°C and 120rpm. Optimum ethanol concentration yield was at 52h. The results are showed that ethanol yield at initial sugar concentration (100%) were (45g/l), at (75%) initial sugar concentration ethanol yield (27g/l), (50%) initial sugar concentration ethanol yield (9.8) and (25%) initial sugar concentration ethanol yield (2.4g/l).

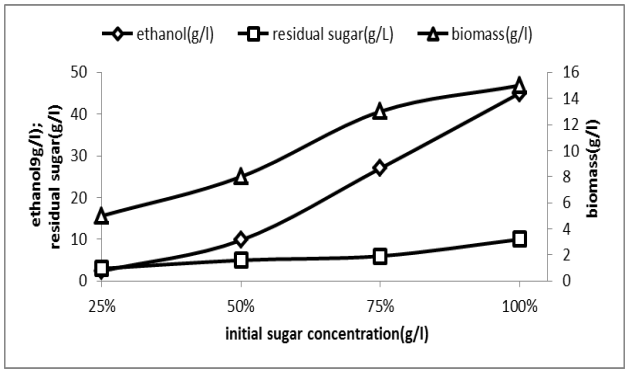

Fig. 10. Effect of initial concentration of sugar on bioethanol production

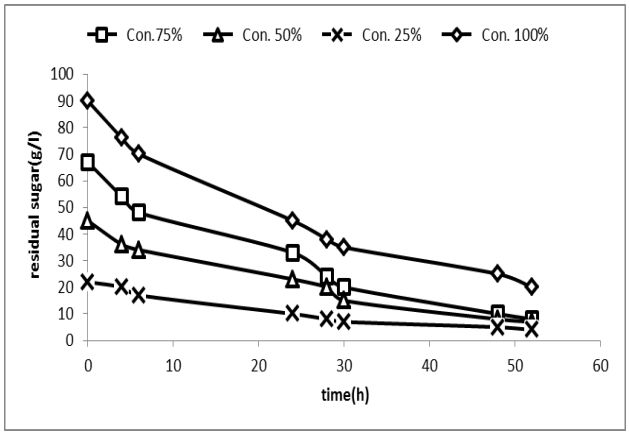

Fig. 11. Consumption sugar during fermentation process during 52hrs

Sugar profiles as well as the ethanol produced during fermentation were shown in Fig (10). The 100% initial sugar concentrations date extract was completely fermented in 28h producing 45g/l ethanol. On the other hand, for initial sugar concentration of date extract of (75, 50 and 25) % resulting in final ethanol concentrations of 27, 9.8 and 2.4 g/l. Lower ethanol yield was obtained for 25% concentration compared to the other two concentrations. It is clear that from Fig (10) higher ethanol yield during fermentation is obtained with date

extract concentration of 100%. This result similar to other study [1]. When compared to biomass growth between four different concentration biomassincreased with high level of concentration as shown in the figure (12). When the fermentation process continued and sugar consumption was continuing simultaneously during the fermentation, and after 52 h, sugar was completely consumed as shown in the figure (11). After consumption of sugar , both the production of ethanol and growth of biomass slowed as shown in the figure (10) as expected these results similar to [20].

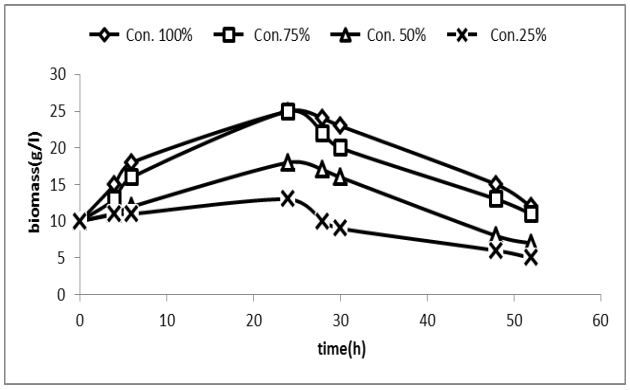

Fig. 12. The biomass in different sugar concentration during fermentation process

4- Conclusion

Low-grade-dates are rich of sugars which can be converted to ethanol and can also attend as essential carbon sources for yeast growth. Other nutrients, minerals and vitamins are also existent within date's constituents which improve the fermentation.

The highest bioethanol yield was 33g/l which obtained with sugar concentration 90 g/l, inoculum size 1%, 52h time and pH-value 5. from initial sugar concentration were most significant factors affecting ethanol production. The batch experiment shows that the bioethanol continues produced during 28h from anaerobic condition, and high bioethanol yield at pH 5 by added 1% from the inoculum size of yeast with initial sugar concentration 90g/l after preparation process.

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انتاج الايثانول الحيوي من التمور رديئة النوعية

رغد علي وحسين فليح

قسم هندسة البيئة–كلية الهندسة–جامعة بغداد

الخلاصة

انتاج الايثانول الحيوي من تخمر السكر واحدة من البدائل المستدامة الحالية عن الوقود الاحفوري.في هذا العمل كان انتاج الايثانول الحيوي من تمور رديئة نوعية غنية بالسكريات حيث يتراوح السكر في التمور رديئة النوعية بمقدار 90غم\لتروالذي يعتبر مصدر اساسي لنموالخمائر في الظروف الهوائية ليتم تحويل السكر الى الايثانول الحيوي في الظروف الاهوائية.هناك العديد من المتغيرات التي تؤثر على انتاج الايثانول منها زمن التخمر,قيمة الاس الهيدروجيني, حجم اللقاح, وتركيز السكر الابتدائي.تغايرت هذه العوامل وذلك لتحديد القيمة المثلى من كل متغير للحصول على اعلى انتاج للايثانول الحيوي. ان اعلى انتاج للايثانول الحيوي هو 33غم\لتر عند 90غم\ لتر من تركيز السكر الابتدائي وحجم لقاح1%,وزمن 52 ساعة,حيث كانت قيمة الاس الهيدروجيني 5.

الكلمات الدالة : الايثانول الحيوي , تخمر , تمور رديئة النوعية , ساكرومايسس سيرفيسيا.