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# Factors Affecting Bioethanol Production from Hydrolyzed Bagasse

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## Abstract

Energy consumption increases steadily as world population increases and concerns about atmospheric pollution derived from fossil fuels have resulted in a worldwide interest in exploring for renewable energy in the form of bioenergy. The conversion of agriculture waste into biofuels, especially fuel ethanol has attracted many researchers. The present study describes ethanol production from hydrolyzed bagasse by a locally isolated yeast strain. There are several factors, especially, fermentation time, temperature, pH, carbon source concentration, nitrogen source and inoculum size, affect on the fermentation process and thus, ethanol yield. To find the optimum conditions. The traditional method for optimization (one variable at a time), was used. The highest ethanol production (61 ml /l) was obtained at 12 h incubation period, 30°C, pH 5, 75% carbon source, peptone as a nitrogen source and 5% inoculum size.

**Keywords:** Bioethanol, Optimization, Yeast, Agriculture waste.

## 1. Introduction

The development of technological society is closely linked to humankind's growing energy needs. Fossil fuels, namely oil, natural gas and coal, have been the fundamental sources of energy during the 20^ th and early 21st centuries. At the same time, they have been increasingly used as raw materials for chemical industries **(Rojas, 2006).**

Presently, we may see that the end of oil exploitation is near. Therefore, future energy generation, as well as fundamental sources of raw materials, will come to rely more and more on renewable sources. Vegetal biomass is a renewable source of energy, chemical products and other materials resulting from the conversion of solar energy by plant photosynthesis. As the end of the "oil age" draws nearer, biomass will play an important role in becoming the base of new industries in the near future **(Abril and Abril, 2009).**

Biofuels include bioethanol, biomethanol, vegetable oils, biodiesel, biogas, biosynthetic gas (bio-syngas), bio-oil and bio-hydrogen. The term biofuels can refer

to fuels for direct combustion for electricity production, but is generally used for liquid fuels for transportation sector **(Balat, 2010)**. Renewable liquid biofuels for transportation have recently attracted huge attention in different countries all over the world because of its renewability, sustainability, common availability, regional development, rural manufacturing jobs, reduction of greenhouse gas emissions, and its biodegradability **(Demirbas, 2008a)**.

The ability to produce biofuels from low-cost biomass such as agricultural waste and by products (including crop residues, sugar cane waste, wood, grass and wastewater from food processing industries) will be the key to make them competitive with other fuels, moreover, only biofuels derived from agriculture wastes show low environmental effects, such as reduction of greenhouse gas (GHG) emission, small land demand and damage to the environment **(Kisielewska, *et al*., 2015).**

Lignocellulosic materials are one of the most abundant natural complex organic carbons in form of plant biomass, which is highly renewable natural resource in the world, reaching annually over 150 billion tons on the earth **(Zhu *et al*., 2006).**

Lignocellulosic materials could produce up to 442 billion liters per year of bioethanol **(Bohlmann, 2006)**. Rice straw is one of the abundant lignocellulosic waste materials in the world. It is annually produced about 731 million tons which is distributed in Africa (20.9 million tons), Asia (667.6 million tons), Europe (3.9 million tons), America (37.2 million tons) and Oceania (1.7 million tons). This amount of rice straw can potentially produce 205 billion liters bioethanol per year, which is the largest amount from a single biomass feedstock (**Karimi *et al*., 2006).**

Egypt is 95% desert and only 5% of the land area is actually occupied with less than 4% of the land is suitable for agriculture. The agricultural activaties result in "the yield" which is economic part of the crop and less important part which used to be called “agricultural waste". Therefore, agricultural waste is defined as the outcome of agricultural production following the different harvesting activities. With the introduction of technology in the agricultural process, waste has become a burden because of the entailed destruction and pollution of the environment. In addition, statistics point out that agricultural waste reaches 30 million tons on the national level **(Shimi, 2005).**

Bioethanol–gasoline blends represent an important role in GHG emissions reduction, urban and road-side pollution and to limit the use of fossil fuels in vehicle engines. Bioethanol is most commonly blended with gasoline in concentrations of 10% bioethanol to 90% gasoline, known as E10 and nicknamed ‘‘gasohol”. Bioethanol can be used as a 5% blend with petrol under the European Union (EU) quality standard EN 228. This blend requires no engine modification and is covered by vehicle warranties **(Demirbas, 2007 and Manzetti, and Andersen, 2015)**

The supernatant from enzymatic hydrolysis of lignocelluloses can contain both six-carbon (hexoses) and five-carbon (pentoses) sugars (if both cellulose and hemicellulose are hydrolyzed). Depending on the lignocellulose source, the hydrolysate typically consists of glucose, xylose, arabinose, galactose, mannose, fucose, and rhamnose**(Keshwani and Cheng, 2009)**. Microorganisms can be used to ferment all lignocellulose-derived sugars to bioethanol.

Microorganisms for bioethanol fermentation can best be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. The performance parameters of fermentation are: temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance**(Dien *et al*., 2003).**

The present study describes ethanol production from hydrolyzed bagasseas a cheap and renewable agriculture waste by a locally isolated yeast strain and studying the optimum condition for fermentation using traditional method to enhance the production of bioethanol.

## 2. Materials and Methods

### 2.1. Microorganisms

#### 2.1.1. Bacterial strain

The bacterial strain MH5 isolated from agriculture waste (bagasse (BA)) and identified by 16s rRNAas *Bacillus flexus* in a previous work. This bacterial strain was used in the present study to saccharify sugar can bagasse (agriculture waste) according to **Abo-state *et al*., 2016.**

### 2.1.2. Yeast strain

The yeast strain MHY1 used in the present study was isolated from agriculture wastes (BA) as previously mentioned by **Abo-State *et al*., 2016**. This yeast strain had been used for fermenting bagasse hydrolysate to bioethanol.

### 2.2. Preparation of bagasse hydrolysate.

According to **Abo-State *et al*., (2013a, b)** and **(2016)** sugar can bagasse (BA)collected from agriculture areas, Upper Egypt. was dried and cut to 3-5 mm lengths, grind in an electric grinder and passed through a sieve to get uniform size. The agriculture waste BA were used as cheap substrate for bioethanol production. Five grams of BA was put in 250ml conical flask and moisten with distilled water. The moisten flasks were sterilized by autoclaving at 121°C for 30 min. The sterilized flasks were inoculated with bacterial strain *Bacillus flexus* (MH5) (2.5 ml of $3.0\times10^5$ CFU / ml). The inoculated flasks were incubated at 30°C for 48 h. After incubation period, 50 ml of distilled water were added to each flask and shacked for 60 min. on shaker (200 rpm). All the content of the flask was filtered in a clean dry flask through muslin cloth on a glass funnel. The filtrate were centrifuged by cooling centrifuge (Sigma, model, 3k30, Germany) at 8000 rpm for 10 min. The filtrate was considered as bagasse hydrolysate which resulted from saccharification of bagasse by the enzymatic activities of *Bacillus flexus* (MH5) via solid state fermentation.

### 2.3. Bioethanol production (Fermentation).

The production medium was formulated according to **Yu and Zhang (2004)**, where peptone (10.0 g/l), $KH_2PO_4$ (2.0 g/l) and $MgSO_4.7H_2O$ (1.0 g/l)(Sigma / Aldrich, USA) were added to bagasse hydrolysate and then sterilized by autoclaving at 121$^o$C for 20 min. The medium was inoculated with 10 % (v/v) yeast strain (MHY1) ($7.7\times10^6$ CFU /ml). The inoculated cultures was incubated at 30$^o$C for 12 h. at 150 rpm in shaking incubator. After incubation, the fermented medium was centrifuged at 10,000 rpm for 10 min. The produced bioethanol and the residual total reducing sugars(TRS) concentrationswere determined **(Abo-State *et al*., 2014).** The ethanol yield was calculated by the modified formula proposed by **Gunasekaran and Kamini (1991)**.

### 2.4. Factors affecting bioethanol production.

The traditional method using change of one factor at a time (i.e. we keep all the factors constant except for the factor to be studied which will be varied) for incubation period samples for bioethanol determination was taken after 12,24, 48, 72, 96 h. under the previously mentioned condition for the fermented medium by yeast strain Y1. In case of studying incubation temperature, the fermented samples were taken after incubation at 20, 25, 30, 35 and 40$^o$C. While studying the effect on initial pH values was determined for fermentation medium adjusted at 4.0, 4.5, 5.0, 5.5 and 6.0 before the fermentation medium be sterilized, inoculated and incubated. For studying the effect of carbon source concentrations, the fermentation mediumwere composed of 10, 25, 50, 75 or 100 % (v/v) of bagasse hydrolysate. The fermentation medium was supplemented with yeast extract (10.0%), Urea (10.0%), ammonium sulphate (10.0%) or ammonium nitrate (10.0%) instead of peptone (10.0%) for studying effect of nitrogen source and the fermented medium was inoculated with 1, 2.5, 5.0, 7.5 or 10.0 ml of yeast strain MHY1 ($7.7\times10^6$CFU/ml) to studying the effect of inoculum size.

### 2.5. Analytical Methods

### 2.5.1.Determination of Total Reducing Sugar

Total reducing sugars were determined by 3,5-dinitro salicylic acid DNS method **(Miller, 1959).** Glucose was used as standard. The samples were stored at 5˚C until analysis to prevent spoilage by microbes and loss of ethanol

### 2.5.2. GC Chromatographic Analysis of Bioethanol

Ethanol production was analyzed by gas chromatography (model 7890, Agilent), equipped with flam ionization detector (FID) and (60 m × 530 µm × 5.00 µm) HP1- capillary column. Helium was the carrier gas, flow rate was 1.5 mL/min. Oven and detector temperature was 300˚C.

## 3. Results and Discussion

### 3.1. Factors affecting bioethanol production

The factors affecting fermentation process for production of ethanol from fermentable sugars derived from agricultural waste using the most promising yeast isolate MHY1have been studied separately each at a time (one variable at a time) as shown in Figures 1-6.

Hydrolysate was obtained from bagasse hydrolysis by bacterial isolate MH5 (*Bacillus flexus*) through a solid state fermentation process, it contained 11.053.68 g/l reducing sugars.

#### 3.1.1. Effect of Incubation Period

Figure (1) showed that shortest incubation time 12h shows the best bioethanol production. This suggests that the fermentation reaction is fast and reaches equilibrium in 12 h. The gradual but slight decrease in ethanol concentration with increasing incubation period could be attributed by loss of ethanol by evaporation and/ or consumption of it by the yeast cells as time passes.

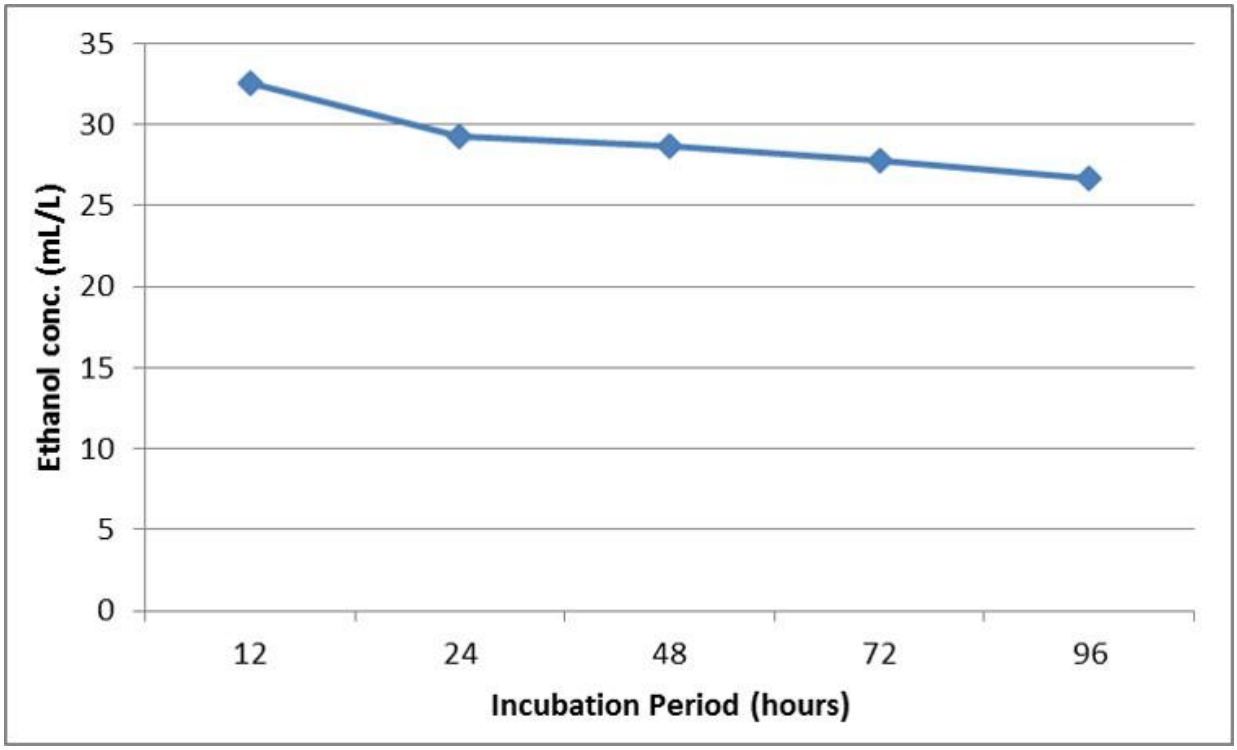


**Fig. (1):** Effect of incubation periods on ethanol production from hydrolyzed bagasse by yeast isolate MHY1.

#### 3.1.2. Effect of Incubation temperature

Temperature has a great effect on the fermentation reaction. As indicated in Figure (2), 30$^{o}$C is the optimum temperature for the fermentation reaction using the yeast isolate MHY1. Below 30 $^{o}$C or above it, the production of ethanol is reduced. Each microorganism has its specific temperature that enhances specific enzymes to catalyze certain required reactions.

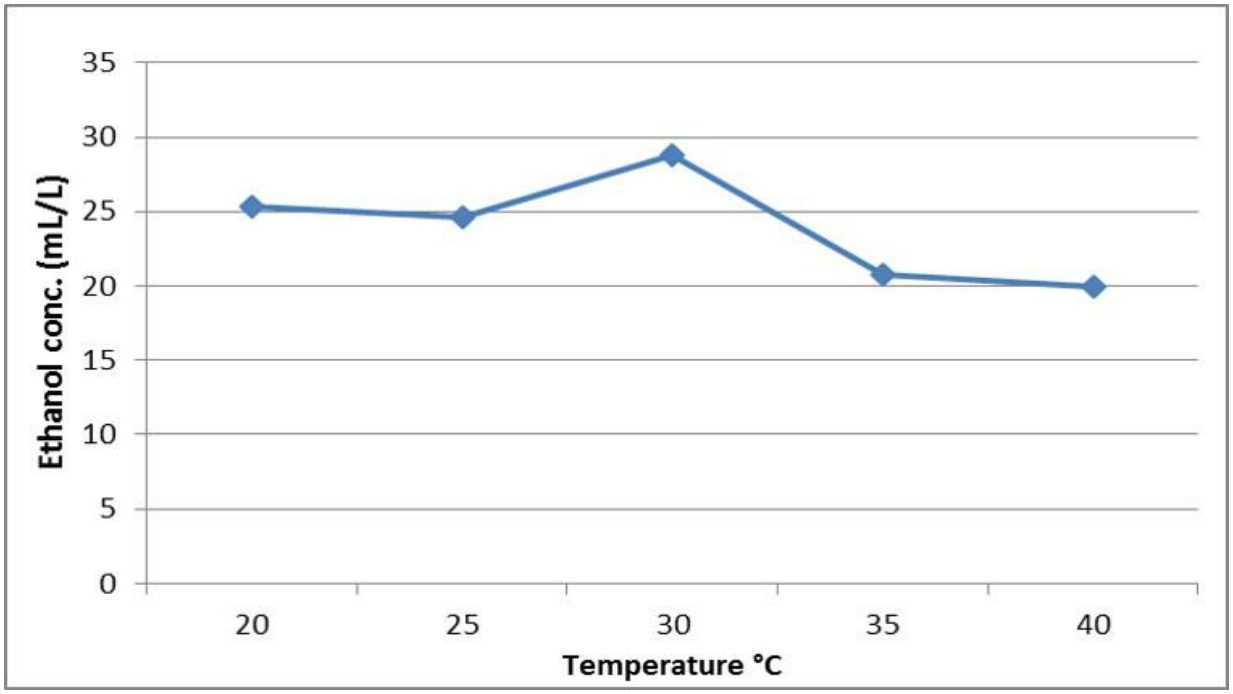


**Fig. (2):** Effect of Incubation temperature on ethanol production from hydrolyzed bagasse by yeast isolateMHY1.

### 3.1.3 Effect of pH

Fermentation reaction was sensitive to changes in pH. The optimum pH value being 5.0 which corresponds to the highest alcohol production as shown in Figure (3).Each microorganism has its specific pH that enhances specific enzymes to catalyze certain required reactions. It is generally known that yeasts favor slightly acidic environment.

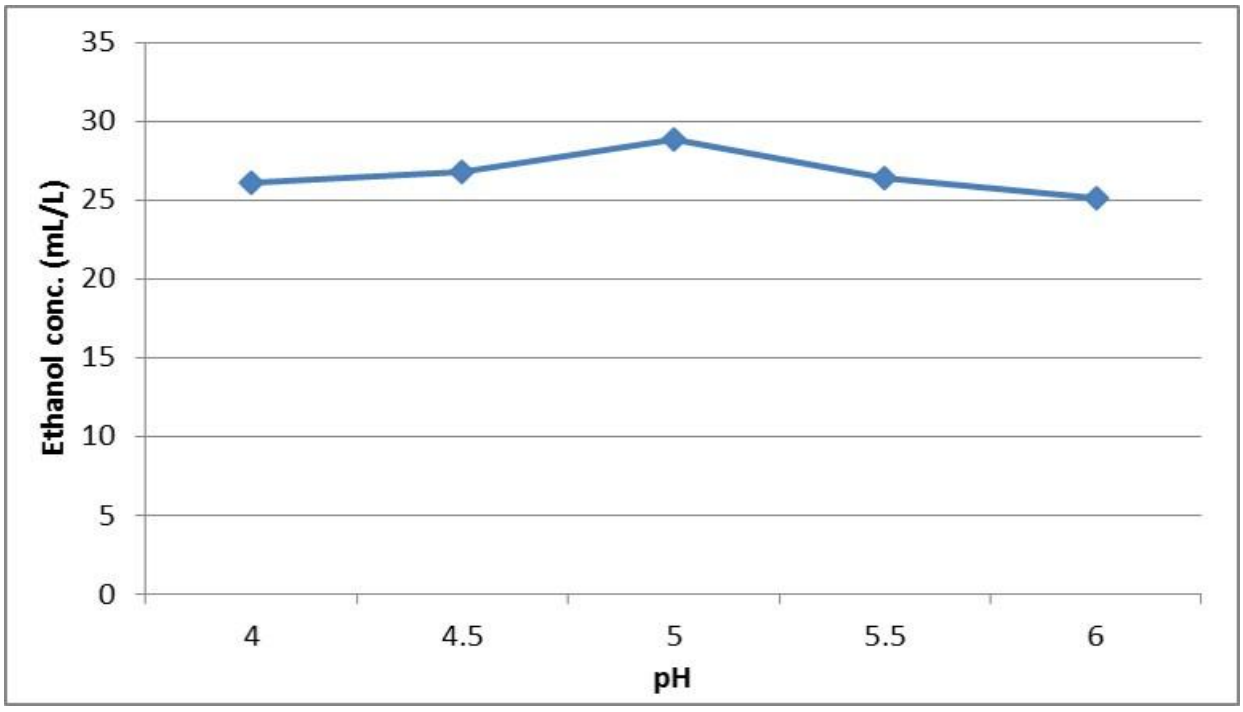


**Fig. (3):** Effect of pH on ethanol production from hydrolyzed bagasse by yeast isolateMHY1.

### 3.1.4 Effect of carbon source concentration

The effect of carbon source concentration on ethanol production using the yeast isolate MHY1 is shown in figure (4). Ethanol production steadily increases with increasing carbon source concentration, reached a maximum at concentration of 75% then decrease. The increase of ethanol production with increasing carbon source concentration was expected since this increase nutrient availability. However, the decrease in ethanol production following a maximum production could be attributed to hydrolysate concentration increases inhibition (as glucose concentration increases) or to product inhibition resulting from initial increase in intercellular ethanol concentration that deactivates the fermenting enzyme or both effects.

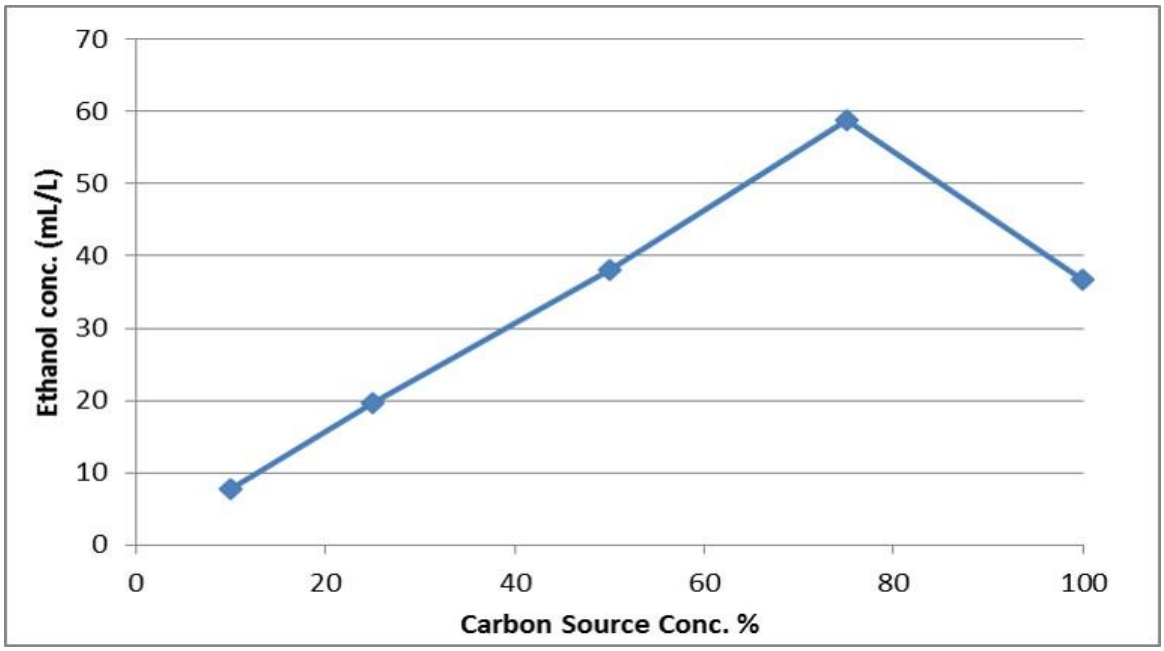


**Fig. (4):** Effect of carbon source concentrations on ethanol from production hydrolyzed bagasse by yeast isolate MHY1.

### 3.1.5 Effect of type of nitrogen source.

According to Figure (5) peptone was be the best nitrogen source for ethanol production using the yeast isolate MHY1.followed by urea, yeast extract, ammonium sulphate and ammonium nitrate in a deciding manner.

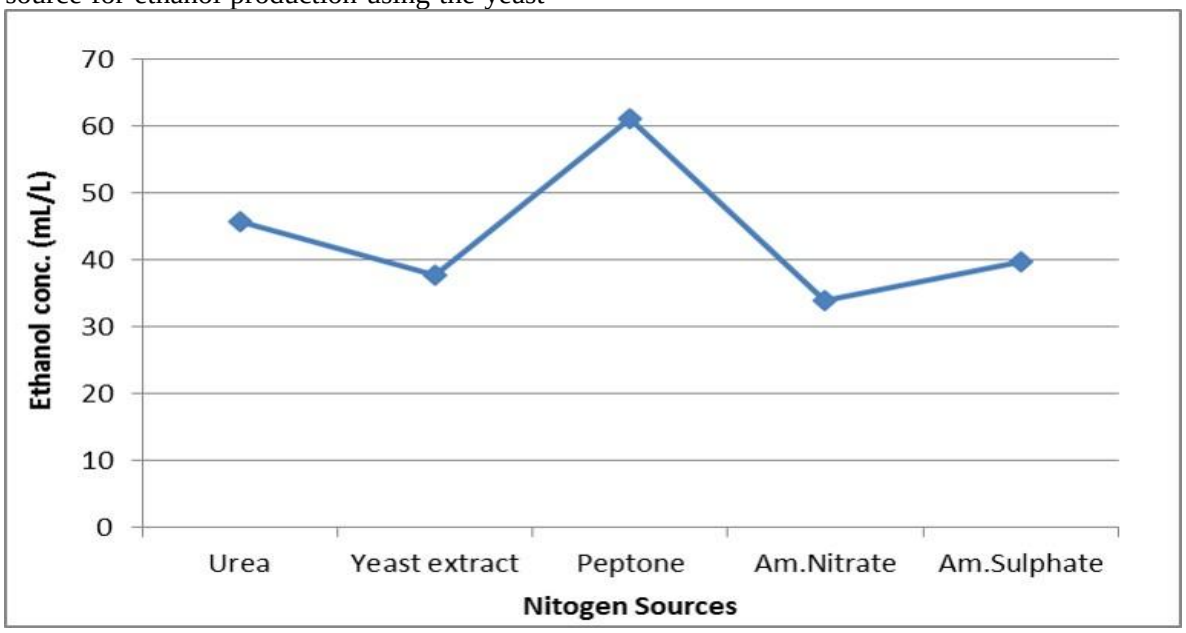


**Fig. (5):**Effect of nitrogen source on ethanol production from hydrolyzed bagasse by yeast isolate MHY1.

### 3.1.6 Effect of inoculum size.

Figure (6) showed the effect of inoculum size on ethanol production using the yeast isolate MHY1. Inoculum size of 5% corresponds to the highest ethanol production rate. Increasing inoculum size beyond this value caused a reduction in ethanol production. Since substrate concentration was the same, initial increase in inoculum size would increase ethanol concentration because of increasing the number of micro-reactors (microorganisms) reaching a maximum of ethanol production, after which, further increase in inoculum size would decrease ethanol production due to depletion of nutrients.

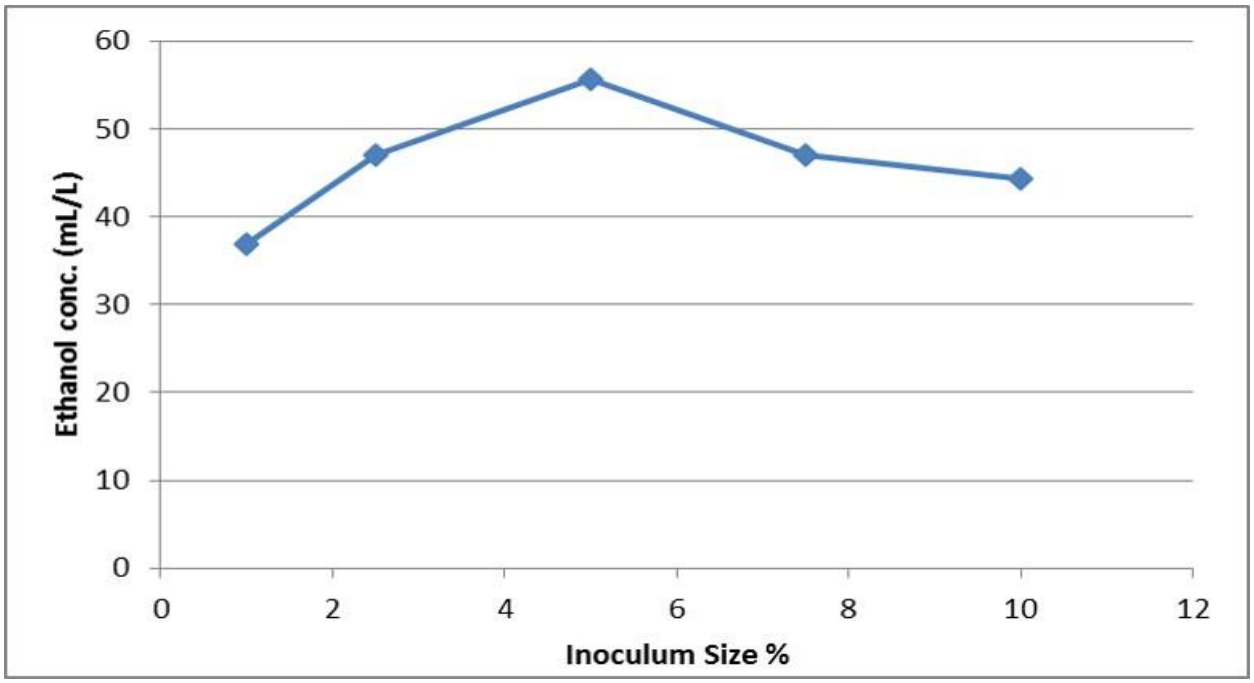


**Fig. (6):** Effect of inoculum size on ethanol production from hydrolyzed bagasse by yeast isolate MHY1.

From the previous results the highest ethanol production (61 ml /l) was obtained at 12 h incubation period, 30°C, pH 5, 75% carbon source, peptone as a nitrogen source and 5% inoculum size using the traditional optimization method.The obtained results were in agreement with results reported by other investigator as the following.

According to **Irfan, *et al*. (2014)** three different substrates like sugarcane Bagasse, rice straw and wheat straw were used for ethanol production by *Sacchromyces cerevisiae* in 500 mL Erlenmyer flask at 30 °C for four days of fermentation period .Among all these tested substrates, sugarcane bagasse (77 g/L) produced more ethanol as compared to rice straw (62 g/L) and wheat straw (44 g/L) using medium composition of (%) 0.25 $(NH_4)2SO_4$, 0.1 $KH_2PO_4$, 0.05 $MgSO_4$, 0.25 Yeast extract by *S. cerevisiae*. This difference in ethanol production was due to the availability of fermentable sugars from cellulose present in biomasses.

**Jalil *et al*. (2010)** used commercial enzyme for saccharification and reported that treated rice straw gave better ethanol production (85 g/L) as compared to untreated (70 g/L) rice straw.

**Uma *et al.* (2010)** pretreated sugarcane bagasse with 1 N NaOH and obtained 48% ethanol production by *C. cladosporoides* after 48 h of fermentation under static condition.

According to **Abo-State *et al*. (2014)**the best ethanol yield was obtained from hydrolysate of *Aspergillusterreus*F98 after fermentation by *S. cerevisiae* Y39 recording 15.25 g/L followed by that obtained with SHF process using *T. viride* F94 and *Candida tropicalis*Y26 recording 12.86 g/L, with ethanol yield of 89.71% and 75.65%, respectively.
**Kumar and Puspha (2012)** reported fungal pretreatment of rice straw by fungal strains *T. ressi* and *A. awamori* in SmF at 5days of incubation at 30˚C produced TRS of 73.7 and 62.7 mg/g, respectively. Ethanol yield after the whole SmF process of 12 days by *Zymomonas mobilis* strain amounted to ≈8.7, 7.9 g/L, respectively.

**Sasikumar and Viruthagiri (2010)** obtained maximum ethanol production (3.36 g/L) from pretreated sugarcane bagasse under optimized process conditions in aerobic batch fermentation.

## 4. Conclusion

The maximum bioethanol production resulted from sugar can bagasse hydrolysated by MH5 bacterial isolate and fermented by yeast isolate (MHY1) by the traditional method was 61 ml/l.

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