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## Bio-ethanol production from Ajowan plant

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### ABSTRACT

Considered to be the cleanest liquid fuel, bio-ethanol can be a reliable alternative to fossil fuels. It is produced by fermentation of sugar components of plant materials. The Ajowan plant is considered to be a favorable source of fermentation products as they have sugar contents as well as contain various nutrients. This study focused on the effective production of ethanol from Ajowan plant by the yeast "*Saccharomyces cerevisiae*" for the first time. The results showed that the total sugar concentration of Ajowan plant was 68.4 g/l. The maximum rate of productivity, ethanol yield and final bioethanol percentage was 8 g/l/h (g ethanol per liter of Ajowan plant per hour), 35 g/l (g ethanol per liter of Ajowan plant) and 76%, respectively.

**Keywords:** Ajowan plant; Bio-ethanol; *Saccharomyces cerevisiae*

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### INTRODUCTION

Due to scarcity of fossil fuel, more and more attempts have been focusing on alternate energy produced by microbial fermentation from renewable sources [1]. Attention has been devoted to the conversion of biomass into fuel ethanol, which is the cleanest liquid fuel alternative to fossil fuels [2]. Biologically produced alcohols, most commonly ethanol, were used as a fuel source for cars well until the 1930s. After World War II, however, little interest remained in using agricultural crops for liquid fuel production because of the abundant and cheap supply of fuel from petroleum and natural gas [3]. With the current limited oil supplies, there is a renewed focus on the need for alternative energy sources; Fuel ethanol remains an attractive option. It benefits farmers by creating a substantial new market for crop supplies and by creating new jobs in economically depressed rural areas and small communities. Ethanol is produced by the action of microorganisms and enzymes through the fermentation of sugars, starches or cellulose [2]. They are widely used in some countries, like Brazil (figure 1).

In the 1970s, Brazil and some other countries have implemented ethanol production from indigenous renewable biomass on a large scale in order to compensate for the rising cost of oil imports. Ethanol is produced by fermentation of the sucrose of sugar cane and *Saccharomyces cerevisiae* yeast. Today, Brazil uses this method to produce 46 percent of the world's annual production of ethanol, about 5/14 billion liters. Failure to generate sufficient ethanol product caused Brazil to obtain its needs via United States and other productive countries [4].

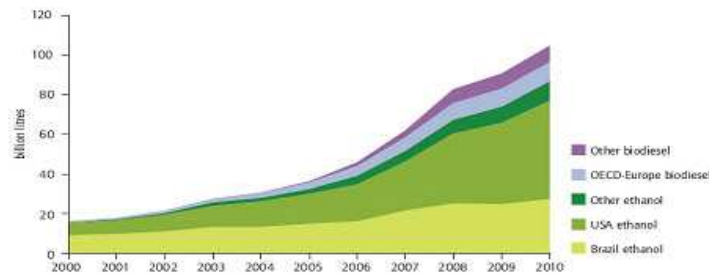


Figure 1. Worldwide production of biofuels by 2010

Apart from of sucrose, other common platforms are available for the production of ethanol, simple sugars derived from plants [5] and dairy waste. Sugar-containing raw materials are usually used directly while starch and cellulose should be hydrolyzed in order to be changed to sugar and produced in ethanol fermentation process(6). Ethanol production process is shown in Figure 2.

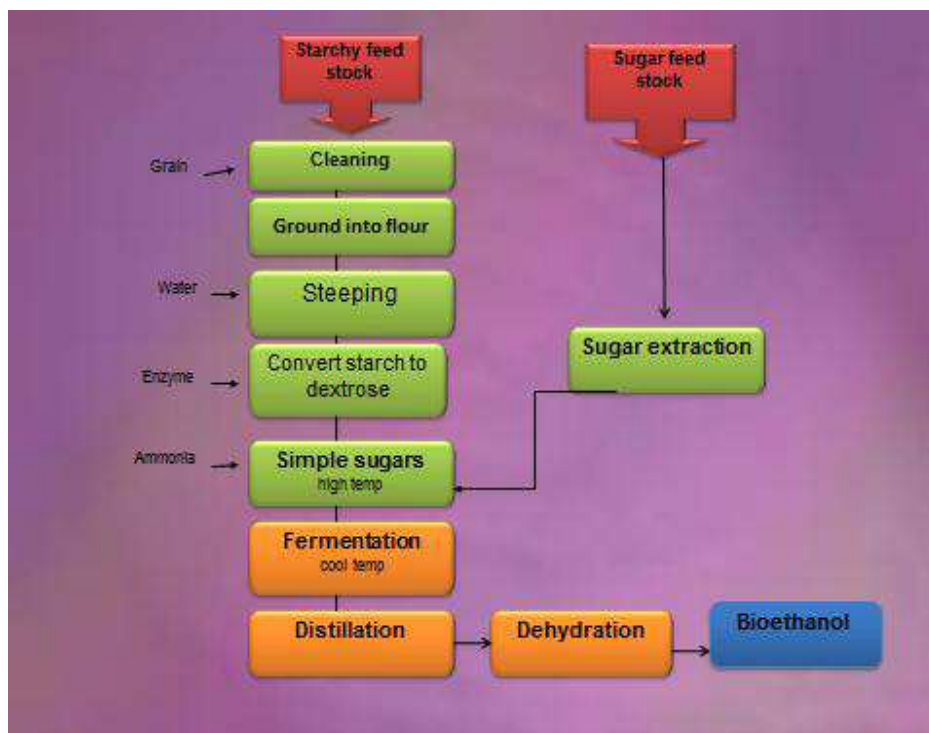


Figure 2. Ethanol production process

In this study, the related substrate is Ajowan with considerable Carbohydrate [7]. In general, all substrates with high amount of carbohydrates are used as ethanol production substrates [8]. *Trachyspermumcopticum* with local pronunciation a: jqu, is actually known as native ecotype of Sistan region that had been used traditionally for medical treatment. Race and type of this plant is Umbelliferae and *Trachyspermum copticum* [9]. It is interesting that it is also known as the Ajwain in Latin too that linguistically is pronounced the same as its Sistani pronunciation(figure 3).



**Figure 3. Ajowan plant**

The traditional use of this plant is to relieve sore throat and pharyngitis in Sistan. Ajowan is also antifungal [10]. Ajowan have antispasmodic, tonic, stimulant and carminative properties. They are prepared in the plasters for pain relief.

Crushed and ground form of this herb is used as an internal medicine to relieve stomach and liver disease, throat problems, cough and rheumatism. Ajowan is famous as enriched source of thymol disinfectant [11]. Ajowan in India is used as a disinfectant, carminative medicine, and tonic to strengthen the stomach and also it is used to stop diarrhea and relieve indigestion, acute abdominal pain or colic treatment is administered [9]. The root of this plant in boiled form is used as a diuretic and carminative medicine. The herb dose in domestic consumptions is equal or less than caraway. It should be considered that hot-tempered individuals should not use it or they should use it as necessary along with cold herbals [11]. This plant grows in the mountains of Khwaja with an area about 30% of the total area mountains.

Bai et al (2008) studied from sugar and starch food in ethanol production technology [5]. Zymomonas ethanol production-based was evaluated from a residual starch hydrolysis by Linda Davis and Hmaran(2006) [12]. Optimization and interactions of medium composition in the production of ethanol was reported using surface response methodology of Zymomonas mobilis by Atompangat(1999) [13]. Vravnkata et al (2006) [14] Vasymvn Ranglk et al (2006) [15] Sonali Patel et al (2008) [16], Yoya Yamishita et al(2008) [17] used some cheap sources like Tapioca(Cassava starch), agro industrial substances, thippi and paper wastes in ethanol production. Linda Davis and colleagues (2006) reported that the current commercial production of ethanol is depended on fermentation of sucrose from sugar cane and molasses or glucose derivatives of starches based on products such as grains, wheat, starches and cassava [12&18].

#### **MATERIALS AND METHODS**

In this innovative project, new substrate characterized as cheap, easy optimization and environmental friend, is replaced by a conventional method. In this method, Ajowan extracts are used for above-mentioned reasons. There is no need to use other nutrients in the medium.

First, both laboratory and commercial species of the yeast *Saccharomyces cerevisiae* were used in YPD medium at 25°C in sugarcane incubator for 24 hours with 120 culture rounds.

Second, optical absorbance of each yeast suspension was at a wavelength of 600 nm.

In the next part of the second stage, optical absorbance of each yeast suspension was reached to 5 by sterile distilled water.

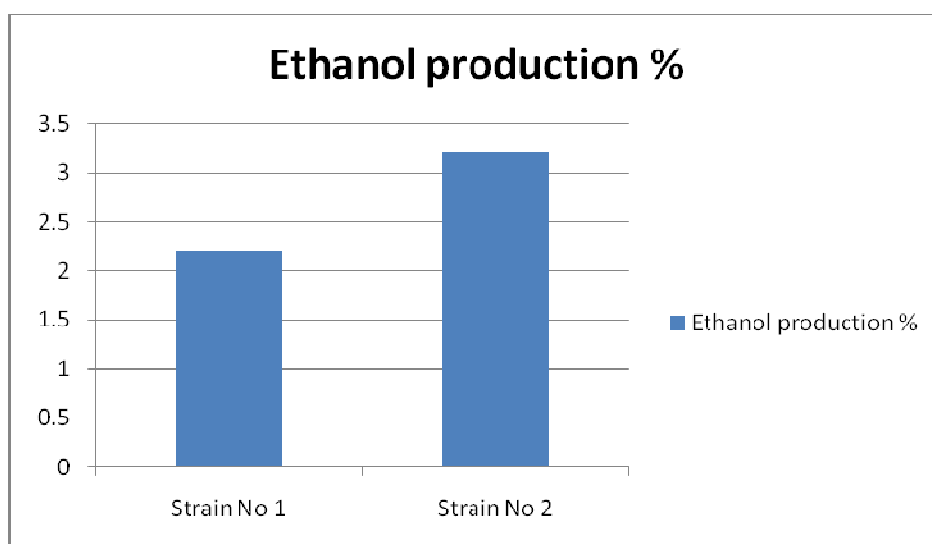
Third, in four sealed flasks at volume of 100 cc, inoculums was added at 50cc and 5 cc in the medium (Extracts after filtration and sterilization by autoclave) that are two flasks in laboratory strains and two commercial strains of *N. Flask*.

Fourth, flasks are placed at 28 ° C for 3 days in sugar cane incubator with 120 rounds.

#### ANALYSIS METHODS:

Samples for biochemical analysis for 6 minutes are put in rpm 7000 in order for rotary. Supernatant obtained for measurement of glucose, ethanol and organic acids was used by HPLC(Tosoh,8020 series) . Analysis of free amino acids were performed by NBD-F technique combined with HPLC [19]. The total amount of glucose was measured by Kalarytm phenol - sulfuric acid [20].

#### RESULTS



Strain number 1 is a genetically engineered strain while number 2 is bread yeast. According to diagram, the bread yeast produced about 3/5% medical; ethanol. Glucose of Ajowan was Extracts 76% . Speeds of ethanol and its value were 8g/l/h and 35g/l, respectively.

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