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Production of Bio-Alcohol Using Yeasts

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Abstract
Most of us know yeasts very useful organisms, especially it is involved in baking, making wine, and industrial ferments. However, yeast is efficient in many areas: like nutrition human, cosmetic, biotechnology, animal health and nutrition, and in fertilization Plants and pest control and have other applications such as biofuel production. Interest increased about the use of fermentation to produce various alcohols and ethyl alcohol in particular and its use provenance energy, especially its use in engines, after the doubling of oil prices during the seventies, where the output of alcohol and its mixing with a certain percentage of gasoline one of the alternatives to conventional fuels. The search for renewable alternatives to permeable fossil fuels is currently underway, and bioethanol is one of these renewable and environmentally friendly alternatives utilized in car engines and others, due to its rapid flammability when mixed with gasoline. The combustion of ethanol results in the release in little quantities of volatile organic compounds such as carbon monoxide and nitrogen oxides. However, the amount of toxic substances resulting from combustion is less than that released from the burning of fossil fuels of fossil fuels such as petroleum, coal and others.
Alcohol produced from various types of substance like sugar, starch, and lignocellulosic by microorganisms such as bacteria and yeasts through three basic stages: the pretreatment, hydrolysis and the Fermentation process. The alcohol production process is influenced by many agents, like temperature, pH, oxygenation, sugar concentration, and nitrogen sources.

Keywords: Yeasts, Production, Bio- Alcohol, Factors Affect

Introduction
Microbial fermentation industries mean the use of microorganisms under controlled anaerobic or anaerobic conditions, to produce useful materials of economic value on a commercial scale, and attention has begun to turn to the importance of the role that microorganisms play in produce of these materials since the studies initiated by the scientist Louis Pasteur on fermentation ″In the second half of the nineteenth century″ when bacteria were used to produce acetone and butanol during World War (. Demirbas, 2009). Fungi and actinomycetes were also utilized to the production of antibiotics during the Second World War, and then there was a great development in the field of microbial fermentation, or the so-called fermentation industries (industrial fermentation), and today there are many chemical and biological materials that are produced by the fermentation industries and many more. Some of them are of great medical, economic and commercial importance, such as the production of alcohols, organic and amino acids, antibiotics, enzymes, vitamins, dextrin and polymers, and from an industrial point of view, microorganisms are a chemical plant capable of causing change. It is desirable in the environment in which you live, with the enzymes it secretes that affect the cheap raw materials that are part of the environment in which they grow and transform them into products of economic importance that are separated and used (da Silva *et al*., 2018). The production of butanol, ethanol, and glycerol is an important alcoholic that are widely produced on a trade level by a wide range of microorganisms such as bacteria, algae and yeasts (Rasul *et al*., 2019).

The Basis for Selecting the Yeasts Used in Production of Alcohol
Yeasts are one of the important organisms used for this purpose, because they produce

alcohol very efficiently with small quantities of by-products, so when selecting them, they must be characterized by the following:

1. The types used must have a high concentration capacity, which allows the use of related media high sugar concentrations.
2. The types of yeasts used should be tolerant of the elevation concentricity of ethanol (Kasavi *et al*., 2012).
3. That ethyl alcohol is the main product to facilitate extraction and purification operations.
4. It has a high growth rate as well as a high fermentation rate, as this helps in the use of brewers small for this purpose.
5. It can live at low pH numbers to reduce pollution (Lin *et al*., 2012).
6. Its optimal for growth should be rather high in order to reduce the cost required for cooling.
7. Be able to grow rapidly.
8. To be of stable hereditary characteristics.
9. To be unsatisfactory (Basso *et al*., 2008).

The appropriate breed can be chosen by one of the following methods:

1. Selection of a strain that enjoys the required specifications from a very large number of isolates, and this is done in the laboratory before starting the manufacture.
2. Make a mutation that contributes to providing the required specifications for isolate.
3. The use of genetic engineering technology to obtain the strain with the desired traits (Mashhor and Mustafa, 2007).

Types of Yeasts Used to Produce of Alcohol

Many yeasts produce Alcohol and the following table shows the most important yeasts produced for alcohol:

Table 1: Yeasts produced of Alcohol

Name of Yeats	Source
Candida auringiensis	Dien, 1996
C. kefyr	Fields, 1982
C. pseudotropicalis	Fields, 1982
C. shehatae	Van Maris *et al*., 2006
C. utilis	Jeffries and Jin,2004
C. tropicalis	Van Maris *et al*., 2006
Kluyveromyces fagilis	Mussato *et al*., 2012
K. lactis	Fields, 1982
K. marxianus	Banat *et al*., 1998
Ogataea polymorpha	Ruchala *et al*., 2020
Pichia tannophilus	Kumari and Pramanik, 2013
Pachysolen sp.	Mussato *et al*., 2012
Pachysolen tannophilus	Van Maris *et al*., 2006
P. stipitis	Selim *et al*., 2018
Saccharomyce cerevisiae	Selim *et al*., 2018
Scheffersomyces stipitis	Ruchala *et al*., 2020
Schizosaccharomyces pombe	Wang *et al*., 1980

The raw material

The substances used to production of alcohol must have many characteristics, the most important of which are: cheap, available locally, rich in carbon source and other nutrients in a suitable way to feed the used strain, easy to store and preferably located near the factory and does not require many and complex processes to prepare it as a food environment suitable for evolution the strains Used (Mashhor and Mustafa, 2007). Raw materials used in the manufacture of alcohol are classified into:

1. Vegetable waste: such as sugar cane, corn, strawberry, and wheat.
2. Hydrocarbons: Some petroleum industries produce gaseous hydrocarbons substance like methane, ethane and propane utilized raw materials (Vohra *et al*., 2014).
3. By-products of food factories: Many by-products and wastes from food factories are used as raw materials, and the following are the most important of these materials:

A. Molasses: One of the by-products of sugar factories, and it is the part that crystallizes from sugarcane juice or sugar beet. Contains 50% sugar (15% sucrose, 35% glucose and fructose).

B. Whey: One of the by-products to working cheese and other dairy foods left behind in large quantities from dairies and their products. It contains about 5% lactose, and it may contain a small percentage of protein and fat, but it may contain some percentage of salt that is added during cheese making.

C. Corn Steep Liquor: One of the remnants of cornstarch factories, which is the water in which the corn is soaked before its manufacture and is rich in various nutrients, and it contains about 6-12% solids materials.

Preparation of the Initiator or Inoculum

it means the active cells that are added to the food medium to start the fermentation process, and the initiator or inoculum in this case is prepared by developing the pure strain from the previous selected yeast and activating it by re-developing it several times on the appropriate nutrient medium under the conditions of sterilization in the laboratory, and the development takes place under aerobic conditions, to encourage the yeast to form large quantities of cells until the size of the inoculum reaches 4 liters, then it is transferred to the production plant to be used as a initiator to produce of alcohol (Mashhor and Mustafa, 2007).

Process in Alcohol Production

There are three main steps in alcohol production

1. Obtain a solution containing degradable sugars.
2. The conversion of sugars into alcohol (ethanol) through fermentation.
3. Disconnection and refining of resulting alcohol (Wyman, 1996).

1. Pretreatment

Substances are treated to reduce their volume and facilitate the subsequent processes, if they contribute to facilitating the process of decomposition and increasing the amount of fermentable sugars (Srichuwong *et al*., 2009). Many methods are used for pretreatment, including physical, such as grinding or chemical, which includes ozonolysis, acid hydrolysis, alkaline hydrolysis (Wang *et al*., 2009) and the organosoly based process (Zhao *et al*., 2009) as well as bioremediation that includes the use of different types of fungi while the physico-chemical treatment includes the use of ammonia fiber explosion (Prasad *et al*., 2007) Steam (Kurian *et al*., 2013).

2. Hydrolysis

The hydrolysis process occurs after pre-treatment to analysis feedstocks into fermentable sugars to produce alcohol. There are two common methods of hydrolysis, acidic and enzymatic, and the ancient widely used method of acidolysis (Jeffries and Jin, 2000). The acid hydrolysis is divided into dilute and concentrated acid decomposition. Dilute acid hydrolysis performed when using high temperatures with a low acid concentration, while the temperature is low when using a high concentration of acid. The dilute acidolysis is more used than the concentrated hydrolysis, but it generates a large amount of inhibitors compared to the concentrate. Acidolysis of lignocellulosic biomass takes place in two stages, as pentose sugars decompose more rapidly compared to hexaccharides. Hemicellulose is hydrolyzed in the first phase is using a dilute acid while cellulose hydrolyzes in the second phase by using concentrated acid is shown to increase the amount of sugars produced to 90% in a little time (Joshi *et al*., 2011).

As for enzymatic hydrolysis, there are three commonly used enzymes for breaking down cellulose: endo-- 1, 4-glucanases, β-glucosidases and cellobiohydrolases. The efficiency of cellulase depends on the concentration and source of the enzyme.

3. Fermentation process

There are three processes used in alcohol: production which are "separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and simultaneous saccharification and co-fermentation (SSCF)" (Canilha *et al*., 2012). SSF and SSCF prefer over SHF because the process can be carried out in the same tank. The benefits of both operations are reduced cost, increase ethanol yield and short treatment time (Chandel *et al*., 2007).

Fermentation Systems to Produce Alcohol

There are two fermentation systems to produce alcohol:

1. Batch Fermentation

In this system, the yeast cells (initiator) are added at the rate of about 5-10% to the fermentation solution (known as Mash) after preparing it appropriately in terms of controlling the concentration of sugar, pH, temperature, and adding the salts of nitrogen and phosphorous in beginning of fermentation process, and the Mash is left in the fermenter. With yeast under anaerobic conditions, after 48 hours the fermentation process ends and the alcohol is extracted (Hadiyanto *et al*., 2013; Thatoi *et al*., 2016). There are many benefits of the batch system involving whole sterilization, not require labor skillfulness, easy to manage the feedstocks, can be can be control easily, and elastic to different produce specifications (Ivanova *et al*., 2011; Jain and Chaurasia, 2014). However, the productivity is lower and need intense and elevation labor costs. The being of increasing sugar concentration in the fermentation medium may lead to substrate repression and results in the inhibition of cell growth and ethanol produce (Cheng *et al*., 2009).

2. Continuous Fermentation

In this system, sugar solution, foodstuffs, and all the inputs are added at appropriate concentrations in form of continuous stream, at a fixed time rate, accurately calculated and not linked to a specific time. In this system, all fermentation conditions such as temperature, pH and ventilation are controlled automatically (Kang *et al*., 2014).

Alcohol Extraction

After the end of the fermentation period, the alcohol is obtained by distillation, through which we can obtain a purity of 95%, but the alcohol will contain a certain percentage of water. Water from alcohol, where regular alcohol is used with a purity of 95% as a solvent and in the pharmaceutical and chemical industries, while very pure alcohol is used 99.85% for special laboratory purposes or for some types of fuels (Mashhor and Mustafa, 2007).

Factors Affecting Alcohol Production

Temperature, pH, oxygenation, initial sugar concentrations, and nitrogen sources are among the most important factors affecting yeast production of alcohol (Lin *et al*., 2012).

1. Temperature

Temperature highly influence on the activity of enzymes and yeast cell membranes, and yeasts that are active and tolerate high temperatures are ideal in produce of industrial alcohol (Ortiz-Mu˜niz *et al*., 2010).

2. pH

Alcohol production is affected by the pH of the medium, the pH is set to 4.5. This is tolerated by the yeasts used in production, and at simultaneously it prevents the growth of many contaminated bacteria whose growth is not suitable for this level of acidity.

3. Oxygenation

The fermentation process takes place under anaerobic conditions until the sugar is efficiently converted into ethanol alcohol and carbon dioxide, so anaerobic conditions must be present during the fermentation process. Usually the yeast cells begin to consume the dissolved air in the solution first, then the anaerobic conditions begin to prevail afterwards and the sugar begins to convert to alcohol instead of producing the yeast cells.

If the availability of anaerobic conditions and the leakage of air into the fermenter do not rule, the yeast tends to multiply and form new yeast cells, and this is at the expense of quantities alcohol formed.

4. initial sugar concentrations

Adjust the sugar concentration to 12%, as the concentration may cause the process to slow down, and at least that is uneconomic and costly (Mashhor and Mustafa, 2007).

5. Nitrogen Sources

The supplementation of exogenous nitrogen provenances like peptone, malt extract, yeast extract, and $(NH_4)2SO_4$ to the natural growing media foster ethanol produce **(**P´erez-Carrillo *et al*., 2011; Harde *et al*., 2014).

The fermentation process to complete the conversion of sugar to carbon dioxide and ethanol takes about 48 hours if the optimum conditions for production are controlled (Mashhor and Mustafa, 2007).

Alcohol Features

Alcohols, such as mainly ethyl alcohol, as well as methyl alcohol, have several beneficial features, including:

1. Alcohol production from renewable materials such as plants, which in turn rid the environment of waste agricultural products that cannot be used directly or indirectly.

2. The combustion of alcohol does not leave any kind of residue and thus it protects the environment from pollution (Guti´errez-Rivera *et al*., 2012; Ishola and Taherzadeh, 2014).
3. Availability of the technical facilities for conducting these fermentations.
4. The alcohol extraction process is easy and inexpensive (Ajanovic, 2011).

Industrial Uses of Alcohol

1. It is used for cooking, lighting, heating and steam production purposes.
2. It is utilized fuel for cars and engines after being mixed with some materials.
3. It is utilized disinfectant and kill germs.
4. It is utilized anti-freezing agent for some purposes (Tesfaw and Assefa, 2014).

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