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# STUDY OF MICRO NUTRIENTS AND CO-CULTURING OF *Saccharomyces cerevisiae* FOR ETHANOL PRODUCTION FROM VARIOUS BIOMASSES: A REVIEW

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Abstract- The aim of the present review is to highlight on some points on the process of fermentation of Bioethanol by using *Saccharomyces cerevisiae* and different biomass such as lignocellulosic products, agricultural wastes as well as paper waste. Different types of Yeasts strains are used in the industries for various purposes such as Baking and Brewing. Beside this to increase the productivity of ethanol and biomass yield co-culturing of *Saccharomyces cerevisiae* with different microbes are discussed. In addition the effect of Metal ions such as Copper, Magnesium and Manganese on yeast growth is also studied in this paper.

Keywords- *Saccharomyces cerevisiae,* Fermentation, Ethanol, Co-culturing, Metal ions, Yeast

## I. Introduction

### 1.1 *Saccharomyces cerevisiae*

*Saccharomyces cerevisiae* commonly known as Brewer's Yeast or Baker's Yeast, is a type of a yeast which was discovered in Malt in 1837. It is the most common yeast which is used in Fermentation. Over the past decades, it has been widely used in making Bread, making Beer and in wine making processes. It is also used for the industrial production of some biopharmaceuticals. It is a unicellular organism, unlike most other complex organism (Eukaryotes), and can be grown on defined media under controlled parameters. The cells of *S. cerevisiae* round to ovoid, and they are about 5-10µm in size. [1] This species is the main source of Yeast Extract and Nutritional yeast. The optimum temperature for growth of *S. cerevisiae* is 30–35 °C (86–95 °F). [2]

## II. Production of Ethanol from different Biomass

The heart in ethanol production process is fermentation, and the process of fermentation is carried out by variety of microorganisms such as yeast fungi and other bacteria. Among all the microorganisms the yeast Saccharomyces cerevisiae is the most widely as well as broadly studied at both industry as well as household levels and this yeast is also generated as ethanol main fermentation product. *Saccharomyces cerevisiae* has evolved with humans since thousands of years in food and beverages as well as research and has become important in various biological activities as well as various other ways specifically ethanol production as a biofuel and hence *Saccharomyces cerevisiae* act as future answer to fossil fuel problem.

Recombinant of *Saccharomyces cerevisiae* were made to yield high value ethanol by protoplasmic fusion by 25 day old palm wine plant because of their high ethanol tolerance .This fusion was done to maximize the profit as well as to improve the strain that may improve both yield as well as product recovery. A variety of organisms were found in palm wine species plant with *Saccharomyces cerevisiae* being the most important in palm juice fermentation that will improve the ethanol yield as well as capabilities of *Saccharomyces cerevisiae* of palm wine origin by protoplast fusion. [3]

Being the most remarkable fuel, ethanol production by *Saccharomyces cerevisiae* has been increasing for ecological as well as economic reasons offering a large scale energy. Lignocellulose is found as one of the major source to produce bio-ethanol. The use of agricultural, industrial as well as various urban wastes can be used for ethanol production which will be eco-friendly as well as profitable. [4]

Lignocellulosic materials has the potential to be a valuable substitute for gasoline. This paper deals with the economic as well as valuable evaluation of process that leads to the production of ethanol from various renewable sources and to produced sustainable energy in order to encourage greater use of bio-fuel from agricultural as well as forest residues. [5]

As we know that ethanol can be produced from various wastes materials having high starch content as well as cellulosic material. This paper deals with the high amount production of ethanol by increasing the barley and yeast amount from different starch sources. It was noted by this paper that various dilutions of acids as well as economical sources of starch should be tried on cellulosic digestion for bio-fuel production to withstand the energy cries. [6]

In order to decrease the stress on environment as well as food prices, the non-edible biomasses can be used for ethanol production. This paper deals with the current as well as future prospect that will lead to bio-ethanol production. Currently for bioethanol productions tremendous contributions has made through distillers in India. Government is also supporting by giving availability to produced ethanol as well as blending it with petrol. So looking at India's fast growing economic, the nation is looking for alternate renewable fuel. [7]

Bioethanol can be produced by acid hydrolysis and subsequent fermentation from office wastes using *Saccharomyces cerevisiae*. The best results were obtained for acid hydrolysis of waste paper with the optimum conditions. This feedstock results to be attractive for biofuel production due to its availability. Since the quality of the paper decreases with the recycling process that is why waste office paper is suitable raw material for obtaining bioethanol. [8]

## III. Yeast

Yeast are eukaryotes, single -celled micro-organisms that are member of kingdom fungus. Yeast are unicellular organisms having multicellular ancestors. The use of yeast cells are very traditional in the fermentation process where cereal mashes, grape must and other naturally derived substrates are used. The use of yeast in production are brewing, baking, wine making and distillation. Also, the substrate provide a great source of carbohydrates, utilized nitrogen, vitamin and other growth factors including minerals. There are various factors that affect the yeast biotechnology that are regulate nutrition, growth, stress response and metabolism in cells. [9]. There are no of number of species of yeast from those one is *Saccharomyces cerevisiae* which converts carbohydrates into carbon dioxide and alcohol. From thousands of years these are used in baking and alcoholic beverages respectively. [10] One other species *Candida albinos* that is a pathogen, causes infection in humans. Now a days, yeast is used for generating electricity and producing ethanol as biofuel. [11] Yeast can be used in number of field of biotechnology and carries a very useful properties. The yeast is oldest techniques used for conversion of sugar via fermentation. According, to the uses of yeast there are various types of that:

- Baker's yeast that is used in bread production.
- Brewer's yeast that is used in beer formation/ fermentation, etc. [12]

### 3.1 Types of Yeast:

#### 3.1.1 Baker's Yeast:

It is active form of yeast. The common function of this yeast to bake bread and various bakery products, which are help in converting the fermentable sugar that is present in dough into carbon dioxide and ethanol. [13] The sugar present in dough will dehydrate it and provide food for growth of yeast. Whereas, the yeast growth is inhibited by both salt and sugar but more by salt. Also, sources like fat, butter or egg just slow down growth. [14]. Today, bees brewers slowly switched from top fermenting to bottom fermenting yeast and this create a shortage of yeast for making bread, so the Vienna process was developed in 1846. [15] Although, there are different form of baker's yeast but the difference comes under moisture content. From all the dry yeast is more preferable due to its long-term storage capacity at room temperature. [16] Some commercially available baker's yeast form are:

a) Compressed yeast: Also called cake yeast. It is available in bulk form for bulk usage. It has poor keeping property so, not commonly available in supermarket. [17].
b) It is not available in small bakery shops but used and can be available in industrial purpose.

c) Active dry yeast: It have coarse granule of yeast, which are encapsulated in thick jacket of dry, dead cells with some growth medium. The advantages is that they can be store at room temperature for years.
d) Instant yeast: These have additionally ascorbic acid although same as dry yeast. [18].

### 3.1.2 Brewer's yeast:

These are inactive form of yeast. This is made of strain chosen for the ability to produce alcohol and have a bitter flavour. The inactive yeast the have yeast in dead form not in alive form. The brewer's yeast have very finite uses such as wines, beers and alcohol beverages industry. [19] According to the historical background of this yeast it can be used to treat various disorders like eczema, nervous disorder, lower cholesterol, treat burn, diabetes, diarrhoea, increase energy and immune tolerance capability, relief stress, detoxify skin, heal wound and reduce wrinkles whereas, no scientist claim about it. Some pet holders sprinkle this yeast in pet's food to save them from flee and ticks. [20]
The brewer's yeast is famous only for alcohol production but it can be a great nutritional supplement that does not mean that it can be a nutritional yeast. But provide vitamin B complex as well as vitamin and minerals which include calcium, potassium, iron and selenium. It also have a rich source of chromium. [21]
The second by-product which is majorly used in brewing industry is *Saccharomyces yeast* biomass. The Brewer's yeast not only used as production of alcohol but can be used as detoxifying effluents which contains heavy metal [22].
The production of beer is not easy it can goes under various processes like chemical and bio chemical reaction such as mashing, boctins, fermentation and maturation. Also, there are three solid-liquid separation for beer production they are wort separation, wort clarification and rough beer clarification. [23]
At the time of fermentation of cooler wort yeast convert sugar to alcohol. The quality of beer has a very great impact of yeast. When the convenient of beer take place there not only fermentation of CO2 and ethanol take place but other compounds also form such as higher alcohol, org. acid, and ether, aldehydes, ketone and sulphur compound that all play a crucial role on the profile of beer. [24]

### 3.1.3 Top yeast:

When the fermentation process is processed than the hydrophobic surface causes the flocks to adhere to carbon dioxide and rise to the top of the fermentation vessel that is termed as top fermenting yeast. Top yeast are hydrophobic in nature. The electrical and electrophoretic mobility is determined by the balance of protonated amino acid and carbohydrate group in protein that gave a high isoelectric point (pH 4) and have a great electrophoretic mobility with pH range of 2 to 7. [25]
The great example of top yeast is *Saccharomyces cerevisiae* that some time called an "ale yeast". [26] Top yeast is formed at higher temperature as compared to bottom yeast. Top yeast have a wilder characteristics that it has tendency to form spores easily, and have great endurance under unfavourable condition such as influence of drying, autolysis, etc. The optimum temperature for top yeast is 95°F, if fermentation carried on this temperature the fermentation process is carried and quality of beer is improved. Although, low temperature is also bad for the quality of beer. So, preferred temperature is in between 68-70°F. Not only temperature but optimum pH value is also required for finished top fermentation of beer that are 3.85-4.15. If the value increases the fermentation reaction become more acidic and the isoelectric point of certain protein in yeast are approached. This result in precipitate in beer and give rise to persistent danger of after precipitate in the beer. There are various factors that are responsible for production of beer in top yeast but there are some factors that are responsible for influence of pH in wort that are carbon dioxide, inorganic salt, protein, amino acid and hop resin. The degeneration of cells can make changes in top yeast. In industries preparation of brewery is same but the difference only in the point of keeping yeast in suspension in the wort.

### 3.1.4 Bottom yeast:

Bottom yeast are generally less hydrophobic in nature because of their higher surface protein concentration. Bottom yeast possess higher surface phosphate concentration. The electrical property mainly control by phosphate, that result in low isoelectric point (pH 2 or below) and an electrophoretic mobility that did not become much more negative above pH 4. [25] The yeast that is produced during the time of fermentation on the bottom of fermenting vessel is termed as bottom yeast. These type of yeast ferment at low temperature. Bottom cropping yeast is *Saccharomyces pastorianus,* formerly known as *saccharomyces carlsbergensis* [26]. Bottom yeast weigh heavier than Top yeast when both are dissolved to make a solution. For bottom yeast optimum temperature is 72°F, if fermentation is carried on this temperature then the quality of beer is increased and also encourage to develop other essential microbes.

## IV. Coculturing of *Saccharomyces cerevisiae* using different microbes

Bio-degradation of aromatic compounds or Biological reduction of sulphates is usually done by a co-culture. A Co-culture is a mimic of a natural environment and is a potential bio-process if there are no cross-interactions among micro-organisms and also micro-organism that is metabolizing the substrate is unaffected by the presence of other micro-organisms. For production of Bio-Fuel from Biomass *Saccharomyces cerevisiae,* an Ethanol producing micro-organism and *Acremonium cellulolyticus,* a hyper cellulose producing micro-organism were co-cultured. The reaction was carried in a single reactor which resulted in production of Ethanol from the cellulose produced simultaneously in the reactor. [27]

In case of dilute acid pre-treatment, it produces furfural and HMF as fermentation inhibitory compounds. *Saccharomyces cerevisiae* have been Co-cultured along with *Pichia stipitis* CBS6054 effectively to convert glucose and xylose into Ethanol in presence of Furfural and HMF. Glucose along with furfural and HMF was consumed in approximately 12hours and xylose was consumed in approximately 96hours giving a higher concentration and higher yield of Ethanol as compared to the coculture of *Issatchenkia orientalis* and *Pichia stipitis* CBS6054. The results clearly demonstrated that co-cultures of *S. cerevisiae* and *P. stipites* were more effective in glucose and xylose consumption and Ethanol production. [28]

The coculture of *Saccharomyces cerevisiae* and *Pichia caribbica* gave the highest ethanol concentration, the lowest levels of residual glucose and fructose, and the highest volumetric productivity of ethanol. [29]

Fermentation of Starch into Ethanol, its amylolytic activity, and Ethanol yields were relatively less in monoculture as compared to the coculture of *Aspergillus niger* and *S. cerevisiae.* Increasing inoculum of *S. cerevisiae* in the coculture increased many-folds of Ethanol production. The results clearly states that the coculture of *Aspergillus species* and *S. cerevisiae* gave much effective simultaneous fermentation of starch into Ethanol than mono-cultures. [30]

*Saccharomyces cerevisiae* was found to be co-cultured with Ragi tapai to study the Ethanol production from unhydrolyzed raw Cassava starch. Ragi tapai is known for its ability to produce higher yields of glucose and ethanol yields from starch. When Ragi tapai was co-cultured with *S. cerevisiae* using the unhydrolyzed raw cassava starch in a single-step fermentation process approximately 46% higher Ethanol yields was found than the monoculture of Ragi tapai in only 24 hours. The level of glucose concentration was kept low in the Coculturing medium. The experiment clearly stated that the coculture of Ragi tapai and *S. cerevisiae* enhanced Ethanol production an also prevented the inhibitory effects of reducing sugars on amylolytic activity. [31]

Generally it was found from various studies that co-culturing of *Saccharomyces cerevisiae* with other different microbes showed increased concentration of Ethanol produced, ethanol yield and production rate. It also resulted in reduction of inhibitory compounds in lignocellulosic hydrolysates, reduction in process cost and fermentation time. Thus, the co-culturing of different microbes in ethanol production could be suggested as a best alternative approach besides the classical way. [32]

## V. Effect of Metal salts on Yeast growth

Mineral salts play an important role in affecting microbiological stability during fermentation process. The effect of various minerals were studied on the fermentation process.

### 5.1 Copper

Copper is a divalent cation which acts as a cofactor for some enzymes such as cytochrome c oxydase, lactase and Cu,Zn-superoxide dismutase. The optimum concentration of copper added in the nutrient media are in the range of 1–10 µM. [33] For many years, copper is known to exert considerable amount of toxic effect on fermentation process and Yeast growth. According to studies it has been found that addition of even minute amount of copper salts in glucose containing media reduced the fermentation process or even terminates the process. Later, it has been found that traces of copper, iron and zinc are essential for yeast growth. [34]

**5.2 Magnesium**

Magnesium is a special enzyme cofactor that is found in most of the biological molecules. It plays an important role in the activation of several glycolytic enzymes such as glucokinase, enolase, and glucose 6 phosphate dehydrogenase) which act as growth enhancer, size transducer and even acts as stress suppressor. [35] It is essential for maintaining Yeast growth and other metabolic functions of physiological importance. The dynamism of Magnesium is affected at the cell wall of yeast cells with Temperature, pH, other mineral ions present and also the viable number of Yeast cells. It has a great role in predicting the performance of Ethanol fermentation. Studies have found Magnesium to have high throughput in regard to Ethanol yield and Biomass yield. In fermentation of lignocellulosic compounds, it has been found that Magnesium produce higher biomass and ethanol yield with Xylose as substrate. Magnesium often exerts positive impact on ethanol fermentation with high substrate concentration. [36]

**5.3 Manganese**

Manganese play an important role in metabolism of yeast S. cerevisiae as a part of some enzymes, e.g. pyruvate carboxylase especially in aerobic conditions. It is present in Golgi and Mitochondria where it activates glycosyltransferases (involved in processing of secreted proteins) and proteases (involved in mitochondrial protein import) respectively. [37] Manganese is required in optimal concentrations for yeast growth. The amount of manganese added to medium is approximately 2-10µM. Manganese transportation in yeast cells is mainly driven by concentration gradient or energy dependent which is stimulated by glucose. Biomass yield and Ethanol yield was increased with the addition of metal ions such as Manganese but it changed drastically with or without the addition of metal ion salts. [33] These experiments show that manganese can affect the fermentation pattern at very low concentrations but do not resolve the conflict over whether manganese is really essential for yeast growth.

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