Methods to Improve Alcohol Fermentation Process: A Review

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Abstract

Biofuel has been a hot research topic in the 21st century because it is promising to gradually replace today's traditional fuel in the future, which can protect the earth from many aspects. For example, biofuel's biodegradability can reduce pollution and solve the problem of the energy crisis to a certain extent. This paper mainly takes ethanol fuel as the research object focuses on the mechanism of yeast production of biofuel, different production methods, and benefits of yeast, and discusses the future of isobutanol, a more efficient biofuel with more significant potential.

Keywords: Alcohol fermentation, anaerobic respiration, ethanol production, biofuel

1. Introduction

With the growth of the idea of sustainable development, the demand for biofuels increase since they cause less pollution to the environment. For instance, the usage of cellulosic ethanol, to its utmost, might reduce greenhouse gas emissions by up to 86% [1], thus slowing down or even improving the situation of global warming. Moreover, due to the particularity of the components of biofuels, the storage and transportation of biofuels will be very safe and convenient, unlike the transportation of traditional fuels such as gasoline, which is extremely dangerous (e.g .gasoline will rock back and forth in the tank truck, resulting in friction with the barrel wall, thus form static electricity and explosion). In addition, biofuel is basically renewable energy, which can truly achieve sustainable development of energy. Moreover, due to its renewable nature, the price of biofuel will definitely decrease in price gradually in the future with proper leading and investments in the field, thus, cheaper and more efficient energy it shall be. Therefore, using yeasts to produce ethanol now becomes a preferable choice for biomass companies, considering that it provides a more environmental-friendly and economical method than the hydration of ethene, which is a chemical way to produce ethanol.

2. Background Information

A fuel made from plant biomass, comprising components from dead organisms and metabolic byproducts of living species, is known as biofuel. A biomass can be converted into biofuels thermally, chemically, and biologically. Biofuels have been utilized for a very long time throughout human history. Contrary to first-generation biofuels, which are made from starch and plant oil, second-generation biofuels are made from lignin materials. This results in a greater difficulty during the extraction of the necessary second-generation biofuel. Therefore, its conversion to liquid fuels needed for transportation involves more numerous physical and chemical treatments.

There is no doubt that biofuels are gradually emerging in today's vision. With the continuous progress of the times, human beings are increasingly discovering the limitations and unsustainability of relying on fossil fuels and other traditional fuels. Nowadays, yeast can plays an essential role in the biomass fuel industry.

The yeasts are fungi with only one cell. Although cells can remain together in brief chains to form a pseudo mycelium, they cannot create real mycelium. The cells might be globose, oval, elongated, or rectangular, and their shapes vary greatly, range from 2 to 8 μ in diameter and 3 to 15 μ in length.

The growth medium and age of the yeast cells (see figure 1), which are highly polymorphic, allow them to take on a variety of shapes. Although yeast cells are hyaline on their own, colonies of them have a white, cream, or slightly brownish appearance. Every yeast cell has a distinct cell wall that surrounds granular cytoplasm, a huge vacuole, and a nucleus. The vacuole's size varies significantly depending on the cell's level of activity [2].

It may occasionally significantly contract, but other than when spores are being formed, it never totally vanishes. Chitin is combined with other chemicals to form a delicate, thin wall. The cytoplasm contains reserve supplies in the form of volutin, glycogen, and oil globules. The cell wall is thin, fragile, and flexible with two layers. Mannan and glucan, two complex polysaccharides, make up the majority of its structure. Protein, lipid, and chitin are present in lower amounts (6–8%, 8.5–10.5%, and, respectively, 30–40%). There is no cellulose. A cytoplasmic membrane or plasma membrane is located inside the cell wall. It encircles the nucleus and cytoplasm. A sizable hyaline structure that takes up a significant amount of the cell and is connected to a strongly stained body on one side are visible under a light microscope. Pores exist in the nuclear membrane. Glycogen, proteins, oil, and refractile volutin granules—an inorganic metaphosphate polymer—contain the cytoplasm in addition to the different cell organelles (mitochondria, endoplasmic reticulum, ribosomes, etc.) that serve as reserve food sources [3].

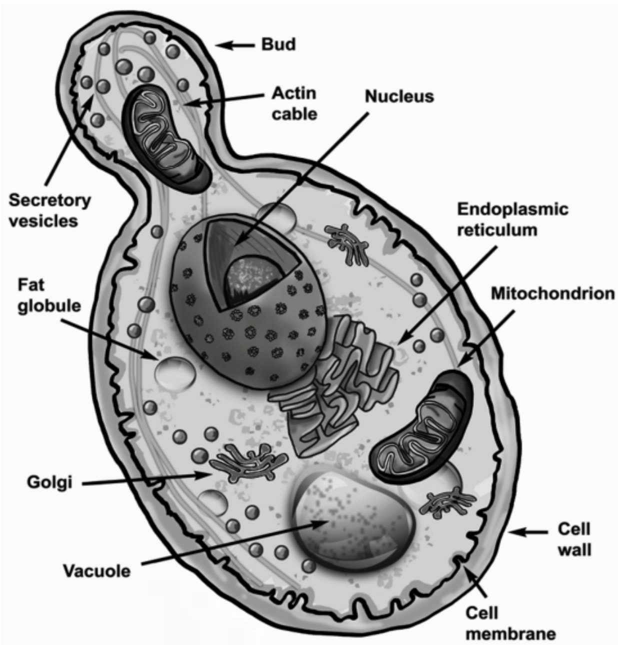

Fig.1. The structure of a yeast cell [4]

Since thousands of years ago, yeast has been engaged in human culture as a biocatalyst. It used to contribute to traditional bakeries and breweries by fermentation, during which fermentable carbon sources will eventually be converted into ethanol. For large-scale fermentations, particular strains of yeasts are usually specific to certain productions. It depends on the required end product, considering different yeast strains have characteristic favours in anaerobic respiration. For example, Saccharomyces cerevisiae is a typical yeast for ethanol production because it has excellent tolerance to pH change. It has been popular in the production of alcohol worldwide.

There are numerous environmental needs for Saccharomyces cerevisiae. The environmental factors could be changed to increase yeast activity. Although it can survive at temperatures as low as 50°C, the best temperature for the maximum specific growth rate and the maximum specific ethanol production rate was found to be between 30 and 45 °C. During the ethanol manufacturing process, Saccharomyces cerevisiae prefers a slightly acidic atmosphere. When the pH value is between 4.0 and 5.0, the likelihood of the ethanol yield reaching its maximum increases. Acetic acid would be produced more readily if the pH was lower than 4.0. The yeast would begin to create butyric acid instead of ethanol when the pH was greater than 5.0. Finally, it needs an environment with an abundance of water action, which assists in maintain yeast metabolism [4].

As well as any organism, yeast also needs particular amounts and types of nutrients to survive. For example, sugars, as an essential energy source. Another one of the most vital substances for saccharomyces cerevisiae is nitrogen, which is primarily taken by active transport [5]. The Yeast Assimilable Nitrogen is mainly in the forms of ammonium salts and alpha-amino acids because of their higher bond polarity. This explains why the uptake of other large proteins and the secondary amine is almost impossible [6]. Besides, certain metal ions like zinc ions are also necessary to adjust enzyme activities. In this case, because zinc ion is an essential cofactor of alcohol dehydrogenase, its absence would cease the entire fermentation. At the same time, some growth factors are also demanded in trace amounts. Those involve certain vitamins, purines, pyrimidines, amino acids, fatty acids, etc. [4].

3. Ethanol Production

Glycolysis and anaerobic respiration are two steps of fermentation. Both of these reactions take place within the cytoplasm. During glycolysis, one glucose molecule is used and two pyruvate molecules are yielded. In this process, two ATP and two NADH are the net products. The two pyruvate molecules are then changed into two acetaldehyde molecules during anaerobic respiration, which also results in the emission of carbon dioxide. Acetaldehyde will thereafter be reduced by NADH produced in glycolysis. Ethanol is the end product [7].

There are several challenges during the production of ethanol. One variable that contributes to the rate of reaction is oxygen concentration. If the O2 concentration is too high, the ethanol yield will immediately decrease since yeasts shift to aerobic respiration. If the O2 concentration is too low, there will be a limited growth of yeast cells. Besides, temperature also controls the rate of fermentation. The high-temperature results in denatured enzymes, while the low temperature will slow down reactions. Although ethanol is the product of anaerobic respiration, a high concentration of ethanol inhibits the reactions. When the concentration of ethanol exceeds 20%, most of the yeast activities will stop [8].

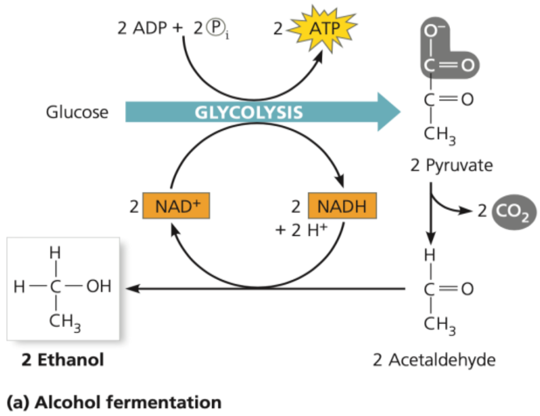

Fig.2. The process of alcohol fermentation [9]

Besides, the efficiency of sugar-ethanol conversion is never completely 100%. One cause of this is that some sugars are taken by contaminated microorganisms. For example, a common contamination of alcohol fermentation (see figure 2), lactic acid bacteria, uses sugars to produce lactic acids. Furthermore, this might be because some of the reactants are used by yeast for producing secondary fermentation metabolite. Example of this involves succinic acid and glycerol. These unwanted reactions would reduce the final yield of ethanol [4].

4. State-of-the-art Results

Existing methods of improving fermentation involve hybrid strains, continuous fermentation, and using high-yeast-cell-density bioprocess.

Considering that Saccharomyces cerevisiae can only ferment hexose but can not ferment other sugars like pentoses and xyloses, the types of reactants for Saccharomyces cerevisiae fermentation would be limited. This problem can be solved by hybridising Saccharomyces cerevisiae with other yeasts which can ferment pentoses, for example, P. tannophilus, C. shehatae and P. stipitis. Therefore, the new strand of yeasts can ferment more than one type of carbon source at the same time.

As mentioned before, providing optimum conditions for yeasts would enhance yeasts' activities. One method to accomplish this is using continuous fermentation. In this method, fresh nutrient base flows into the culture at certain intervals. Meanwhile, the mixture flows out from the culture at the same speed. This ensures a constant volume inside the culture. Through this process, a stable ethanol yield is more easily to be achieved by adjusting the interval of flow and other conditions. At the same time, since different cultures can be controlled simultaneously, continuous fermentation could support mass production to meet the large demand for ethanol. It is also an economical method. However, there is a higher risk of exposure to contamination. Moreover, the reactants may not be fully used by yeasts because the base continuously flows out, thus causing a waste of resources.

A simple way to improve the ethanol yield is to directly increase the concentration of yeast. This is called high yeast cell density bioprocess, down from which there are two types: local high cell density and global high cell density. As shown in figure 3, the difference between them is that yeast cells in local high cell density are all immobilised in flocculating pellets, whereas yeast cells in global high cell density are "naked". With this method, cells would be less affected by the inhibitors [10].

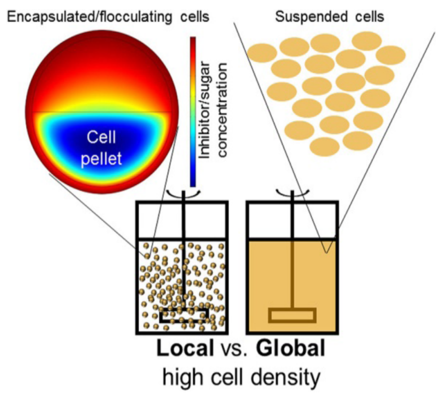

Fig.3. The differences between local and global high cell density method [10]

Yeast immobilization is known as the isolation of biological catalysts to a region of space, which contains reactants, with the conservation of cellular biological activity. Although the immobilization of yeasts provides an access to high-yeast-cell-density production, it may change the growth and metabolism of yeasts in an irregular way, and thus affecting the yield of alcohol [11].

5. Future Directions

5.1 Using Isobutanol

Isobutanol has remarkably significant growth prospects for the possibilities of future alcohol fuel use. Since it has a 25% higher energy density than ethanol, isobutanol is considerably more suitable for use in automobiles than currently available ethanol-based fuels [12]. In terms of fuel, isobutanol also outperforms ethanol in terms of lower volatility and lower hygroscopicity [13]. Isobutanol is thus a promising choice for upcoming biofuels.

But although isobutanol is much more bioenergetic than ethanol, it is also more toxic to yeast cells. So, there's also a burden to increase isobutanol production for its highly virulent nature. At the same time, scientists have discovered a mechanism that yeast cells use to protect themselves. Scientists call it the starvation response. This response illustrates the phenomena that isobutanol signals to a yeast cell that it is starving far before that toxic level is reached, similar to how stomach rumbles inform us we need to eat. When given this information, the yeast stops growing, stops producing more isobutanol, and conserves resources instead of pumping out isobutanol in the large quantities required for commercial manufacturing [12]. Therefore, one of the current research projects focuses on how to modify or screen suitable yeast strains in order to boost isobutanol synthesis.

Princeton researchers have identified a gene (GLN3 gene) involved in the starvation response by yeast to isobutanol. Deleting this gene turned out to profoundly enhance yeast's tolerance to the chemical. Freed of thinking it's missing meals when it's not, the yeast reallocated its resources to staving off toxic effects from higher-than-usual isobutanol concentrations. Generally, the genetically modified yeast cells produced five times as much isobutanol as unmodified yeast, marking a significant advancement in the production of sustainable biofuels as part of the multifaceted approach to reducing the effects of global warming [12]. The current study's conclusion raises the possibility that gene editing could enhance isobutanol synthesis.

Another method of increasing production rate using the yeast Saccharomyces cerevisiae is to successively block the generation of ethanol and glycerol as well as competing metabolic pathways [14]. A preliminary result has been achieved through relevant field research. We were able to produce isobutanol with a yield of 59.55 mg/g glucose, which is the highest yield ever with S. cerevisiae in shake flask cultures, by overexpressing a cytosolic isobutanol synthesis pathway and by blocking non-essential isobutanol competing pathways, according to Johannes Wess, Martin Brinek, and Eckhard Boles. However, our findings show that the isobutanol synthesis route itself still has a restricted capacity [14].

Therefore, in general, the development prospect of alcohol fuel is very broad, for the future development of human beings also has a very important role, but the existing level of science and technology is still difficult to replace traditional fuel with alcohol fuel in a short period of time, a variety of methods still need to be explored.

5.2 Yeast Immobilization

The immobilization of yeasts has an impact on many yeast cells characteristics, which involves viability, metabolism, and toxin resistance. In some experiments, when yeast cells are incorporated in winemaking, some notable changes in the concentrations of metabolites (including ethanol and higher alcohol) are reported. This proves that altering the conditions of immobilization could be a prospective way to adjust alcohol fermentation process.

One system called "yeast biocapsules" claims to minimize the impacts on cells, while still keeping the activity of yeasts. Because yeasts can attach to each other, the demand to extra supports is less needed. Therefore, the capital cost could be lower [11].

6. Conclusion

Based on the certain gains brought by immobilised yeast, we also have a vision to apply immobilised yeast technology to the production of isobutanol, which can reduce the toxicity of isobutanol to yeast cells to a certain extent, and also delay the problem of yeast accepting the signal of isobutanol and thus reducing the yield, which can also be used in brewing, biofuel production and other fields that rely on fermentation. This can lead to significant yield breakthroughs in a number of fermentation-dependent areas, thereby increasing the value in terms of economic efficiency and product qualities.

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