

Submerged and solid state fermentation



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Higher fungi, also known as Dikarya, are a subkingdom of Fungi that is comprised of the phyla Ascomycota and Basidiomycota. They do not possess flagella and can be either filamentous or unicellular. Ascomycota is the largest phylum of the Kingdom Fungi, it contains over 64,000 species. The ascomycota classification makes up more than 75% of fungi. It is distinguished by a sexual structure called an ascus, meaning sac, inside which ascospores are formed. The ascomycota fungal group all belong to a common ancestor which means they are a monophyletic group. The ascomycetes are useful to humans as they are used in making cheese and bread; they are also part of the antibiotic production method. There are many known ascomycetes for example truffles, *Saccharomyces cerevisiae* (baker's yeast) and *Penicillium chrysogenum* (penicillin). Basidiomycota are filamentous fungi and they are made up of hyphae. Basidiomycota is renowned for the production of large fruitbodies such as the mushrooms, puffballs, jelly fungi etc. Basidiomycota are the most evolutionarily advanced fungi. They contain basidiospores which are spores specialized for sexual reproduction. However some basidiomycota can also reproduce asexually. Basidiomycota also contain some yeasts, one of the most common yeasts is *Sporobolomyces roseus*, this contains basidiospores which are respiratory allergens.

1. 2 Fermentation

Fermentation is a metabolic process during which carbohydrates are converted into alcohol and carbon dioxide or other acids. Fermentation is an anaerobic process in which energy is released from glucose without the need for oxygen. Fermentation occurs in yeast cells, they obtain energy by

converting sugar into alcohol. Bacteria are also involved in fermentation; they convert carbohydrates into lactic acid. Yeasts are involved in both bread and alcohol production. During alcohol production, fermentation yields beer, wine, and other spirits. The carbon dioxide produced by yeast activity combines with the carbon dioxide emitted in the Krebs cycle which results in the rise of bread. Another use of fermentation is its ability to preserve foods for example, it produces lactic acid in yogurt, and it is also used in the pickling of foods with vinegar. Fermentation also occurs naturally and has been happening since before human time. However recently fermentation has become the controlled process we know it as today. The study of fermentation is known as zymology. In 1856 French chemist Louis Pasteur became the first known zymologist, when he demonstrated fermentation was caused by living cells. In 1860 he demonstrated that bacteria cause souring in milk, it was previously thought this a simple chemical change. He also successfully identified the role of microorganisms in food spoilage; this resulted in the discovery of pasteurization. While he was investigating the fermentation of sugar to alcohol by yeast, Louis Pasteur found that the fermentation was caused by forces called ferments, which were inside the yeast cells. The metabolising of glucose can occur in yeast cells by cellular respiration. This can also occur in other cells. In the absence of oxygen, glycolysis occurs, which results in the metabolising of glucose into pyruvic acid. This pyruvic acid is then converted first to acetaldehyde and then to ethyl alcohol. The conversion of energy to the yeast cell results in the production of two molecules usually produced in glycolysis. This process is known as the crabtree effect.

2.0 Submerged Fermentation

Submerged fermentation is a process involving the development of microorganisms in a liquid broth. This liquid broth contains nutrients and it results in the production of industrial enzymes, antibiotics or other products. The process involves taking a specific microorganism such, as fungi, and placing it in a small closed flask containing the rich nutrient broth. A high volume of oxygen is also required for the process. The production of enzymes then occurs when the fungi interact with the nutrients on the broth resulting in them being broken down. At industrial level this production of yeasts has become a major output of microbiological industries as a result of improved fermentation technologies. Fermentation in industries is carried out using fermenters which are large vessels which can store huge volumes. In an effort to reduce nitrogen and carbon levels, microorganisms secrete enzymes in the selected medium. There are two common methods by which submerged fermentation takes place; they are batch-fed fermentation and continuous fermentation. In batch-fed fermentation sterilised growth nutrients are added to a culture. It is most common in bio-industries as it occurs during the growth of bio-mass in the fermenter. It helps raise the cell density in the bioreactor and it is typically highly concentrated to stop dilution. The rate of growth in the culture is maintained by adding nutrients, this also reduces the risk of overflow metabolism. An open system is constructed for continuous fermentation. Then sterilised liquid nutrients are slowly and continuously added to the bioreactor at the same rate at which the converted nutrient solution is being recovered from the system. This results in a steady-rate production of the fermentation broth. In order to

maintain a successful fermentation certain variables must be monitored, for example, temperature, pH, as well as oxygen and carbon dioxide levels.

2. 1 Citric Acid Production

An example of submerged fermentation can be seen in the production of citric acid. Every year over a million tones of citric acid is produced by fermentation. In 1893, C. Wehmer became the first person to produce citric acid from sugar by using *Penicillium* mold. However it was James Currie who first established that strains of the fungus, *Aspergillus niger*, could be used to produce citric acid. Two years later the first industrial scaled production of citric acid was started by a pharmaceutical company named Pfizer. This fermentation process is still the process used by industries to produce citric acid today. It is the preferred method for production of citric acid as the probability is low and therefore economical constrictions are in place. *A. niger* is the chosen strain of fungi used at it is used as a substrate and it produces consistently high yields. In the process *A. niger* cultures are placed onto a sugar containing medium, the mold is then removed by filtration and the citric acid is separated using precipitation with lime. Sulfuric acid is then used on the resulting calcium citrate salt to produce citric acid.

3. 0 Solid State Fermentation

Solid state fermentation is a manufacturing process used in the production of fuel, food, pharmaceutical and industrial products. It is used as an alternative to submerged fermentation. It is known in Japan as Koji fermentation and has existed for many years. It is the use of microorganisms in a controlled environment to produce enzymes, fuel and nutrients. Solid state fermentation occurs in the absence of free water. There are a number of

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advantages of the use of solid state fermentation over submerged fermentation. It is a much more simple process which requires a lot less energy. It produces a much higher volumetric productivity and it is similar to the natural environment of certain fungi. The volumetric productivity can be up to eight times greater than that of submerged fermentation. Solid state fermentation also has a more easy downstream process than submerged fermentation. The solid state fermentation process involves a solid matrix like rice bran and placing it on a medium to alongside microorganisms create a substrate. This is then stored in at a specific temperature, between 5 and 95 degrees Celsius, for one to five days. It is also subject to agitation using constant or intermittent rotation. Solid state fermentation plays a key role in developing filamentous fungi. It allows air to come in contact with the mycelium by smearing the mycelium. This is important as filamentous fungi can be decomposed in their natural conditions as they are on the ground. Solid state fermentation allows the growth of filamentous fungi in conditions which represent their natural environment. The growth of mould is promoted by using substrates which have a reduced water level. One of the most common substrates used is wheat bran. It is necessary to monitor the rate of air flow as this has an effect on water and oxygen levels as well as any changes in temperature. Moisture levels are vital for the growth of filamentous fungi and the moisture content must be maintained at a specific level. Sterilisation of the environment is not necessarily required when carrying out solid state fermentation, this is because the fermentation substrate initiates sterilisation and the microorganisms prohibit micro flora from growing.

3. 1 Koji Fermentation

An example of solid state fermentation is koji fermentation. This is Japanese for *Aspergillus oryzae* which is a filamentous fungus (mold). In Japanese and Chinese cuisine *A. oryzae* is used in the fermentation of soy beans. Another use of *A. oryzae* is to saccharify rice as well as potatoes in alcohol production. It is also used to produce rice vinegars. It plays a huge role in making Japanese drinks like sake and huangjiu. High starch ingredients such as rice and manioc are typically used to make sake and other alcoholic drinks as opposed to using malted barley or grapes. These koji molds are specialized to ferment the starch ingredients into simple sugars.

Saccharomyces yeasts cannot break down these starches. It is understood that *A. oryzae* was domesticated up to 2000 years ago. There are numerous properties of *A. oryzae* which make it a vital component of alcohol production and rice saccharification. For example it can secrete amylases and has a small amount of tyrosinase. Another benefit is its rate of growth. The mycelia grow rapidly onto rice kernels for rice saccharification. *A. oryzae* gives off a nice odour and has a range of flavours. It also has a low production of harmful colour substances. The koji fungus has been referred to as “national fungus” because of the impact it has made in Japan for the production of sake as well as soy sauce and other important Japanese foods.

4. 0 Future Prospects

It is evident that both submerged and solid state fermentation are both successful techniques which are used to great effect by higher fungi to produce many valuable products. They do this on both a small and industrial scale. However some of these techniques have now become out-dated and a

new fresh approach may have to be taken with regards to the future perspectives of fermentation used by higher fungi. For me there are a number of reasons as to why solid state fermentation is the way forward. One of these is the current economic situation which immediately means that cost efficiency is a key element in the future. Solid state fermentation offers greater energy efficiency and a lower water consumption than submerged fermentation. Another reason that solid state fermentation is important is because of the increased awareness of protecting the environment. As solid state fermentation has a lower energy consumption it has less ill effects on the environment. It also has a lower production of effluent which therefore decreases the risk of pollution in the environment.

4. 1 Bioreactors

In the last few years there have been major developments in the technologies used in solid state fermentation. The progress in biochemical engineering has led to the breakthroughs in mathematical modelling which is important in determining the cellular growth of microorganisms. Progress has also been made in the designing and development of bioreactors (fermenters). Already heat and mass transfer effects have been overcome when filamentous fungi are growing into the solid substrate bed. Also the packed beds idea in bioreactors would allow unmixed beds to be agitated intermittently or continuously with forced aeration. In the future solid state fermentation could play a key role in bioremediation and the reduction of harmful and toxic wastes in the environment. In the future the aim is to produce a single computer which can control several different solid state bioreactors at the touch of a button. Not only would this provide better

information and control of the bioreactors but it would also reduce operational costs.

4.2 Ethanol Production

Another important sector in the future of fermentation by higher fungi is industrial ethanol production. Yeast has the ability to convert sugars into cellular energy and as a result produce ethanol. The fermentation results in the production of ethanol, alcoholic beverages and the rise of dough. Yeasts which have undergone genetic engineering can play a role in the fermentation of xylose. Xylose is a sugar which is found in many biomass substances. This results in the efficient production of ethanol at much lower costs. It also offers a fuel which can compete in cost with gasoline.

Conclusion

Although I feel both submerged and solid state fermentation processes involving higher fungi are successful and productive there are certain interesting areas which can be explored to further develop efficiency and capabilities. Particularly in solid state fermentation progress can be made in developing new technologies to carry out fermentation. There is a huge potential for the engineering of new bioreactors to improve fermentation. There must be an emphasis put on cost effective techniques as funding has been cut due to the economic downturn. As always these processes must take into account the environmental impact which they have. It is important now more than ever to minimise the release of harmful substances and chemical toxins into the environment. I feel that further research and study into the production of biomasses such as ethanol would be hugely beneficial

to the environment. Overall I feel that further progression can and will be made in both submerged and solid state fermentation used by higher fungi.