

Design, development and comparison of ethanol production from free and immobilized *saccharomyces cerevisiae* under stationary culture

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Abstract

Background: The aim of the present study is to design, develop and compare the production capacity and the difference in the yield of the ethanol by fermentation technology. The utility of the alcohol per annum is increasing day by day as the number of industries using ethanol as a solvent, pharmaceutical aid. Hence the production of ethanol by fermentation technology has triggered us to investigate the best way and method to produce ethanol of benefit of Pharmaceutical Industry. Method: The conventional method of production of alcohol was performed by aerobic batch type of fermentation: the yield was noted. The same production was performed by using immobilized yeast cells by gel entrapment technique. We produced ethanol by free and immobilized *saccharomyces cerevisiae* under stationary culture. Cane molasses was used as a sugar source for about (12 – 21%), medium pH (4-5.5). incubation temperature (25^oc-30^oc), volume of fermentation medium (200 – 350ml) and reuse of immobilized yeast cells. Result: Rate of ethanol production was maximal with immobilized yeast cells. The results indicate that 2gm vegetative cells of yeast on utilizing molasses at 15% sugar concentration with medium pH 4.5 at 30°C. Conclusion: Hence we conclude that the fermentation optimization and the yield value of popular organic solvent (ethanol) was studied and reported. The specific gravity of the distilled alcohol by free and immobilized yeast cells were found to be 0.9730, 0.9705 at room temperature and the alcohol content was found to be 85%, 93.52% as per International Pharmacopeia respectively. To conclude the immobilized yeast cells were found to yield more ethanol than free yeast cells.

Keywords: Fermentation of ethanol, batch type culture, immobilized cells, *saccharomyces cerevisiae*

This is the process for the production of desired product by the mass culture of microorganisms. Biochemical activity of microorganisms where by the development, reproduction, is carried out in a closed vessel for the production of desired valuable product. Fermentation is the application of microorganisms to convert simple raw materials in to valuable products by chemical and biological change.^[1,2]

The industrial usage of microorganisms often requires that they are grown in large vessels are commonly called fermenters and they can be quite complicated in design size frequently they must provide for the control and observation of the many facts of microbial growth and biosynthesis. Therefore before we can consider the development of the fermentation process are the mechanics of existing fermentations we must know something of design and operations of those fermenters and their auxiliary equipment and of the limitations imposed by these fermenters on the ways in which microorganisms can be cultivated on an industrial scale.^[1,2]

These vessels must be strong enough to withstand the pressure of large volumes of aqueous medium but the same time the materials from which they are fabricated must not be corroded by by the fermentation product nor contribute toxic ions to the growth medium fermentations utilize pure culture fermenters must make some provision for the control of or prevention of the growth of contaminating microorganisms. If the growth of the fermentation of the microorganisms is to occur aerobically then provision since most industrial must be made from rapid incorporation of sterile air into the medium in such a manner that the oxygen of this air is dissolved in the medium and therefore readily available to the microorganism and that the carbon dioxide resulting from microbial metabolism is largely flushed from the medium some form of stirring should be available if not accomplished by gas evolution during microbial growth to both mix the organisms through the medium and to make nutrients and oxygen more available to the individual microbe^[3,4].

Immobilization

Immobilization of enzymes for cells refers to the technique of confining/anchoring the enzymes in or an inert support for their stability and functional reuse. By employing this technique enzymes are made more efficient and cost effective for their industrial use. Some workers regard immobilization as a goose with golden egg in enzyme technology.

Immobilization in biotechnology is the used for the physical or chemical fixation of cells, organelles, enzymes, or other proteins onto a solid support, into a solid matrix or retained by a membrane, in order to increase their stability and

make possible their repeated or continued use. Therefore it is expected that the microenvironment surrounding the immobilized cells is not necessarily the same experienced by their free cell counterparts.^[5]

Immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial process. While the attached cells in natural habitat exhibit significant growth, the artificially immobilized cells are allowed restricted growth. Since the time first reports of successful application of immobilized cells in industrial applications, several research groups world over have attempted whole cell immobilization as a viable alternative to conventional microbial fermentations. Using immobilized cells, different bioreactor configuration were reported with variable success.^[6,7] The study on the physiology of immobilized cells and development of non-invasive measuring techniques have remarkably improved our understanding on microbial metabolism under immobilized state. We have presented an overview of this field.

Immobilization techniques used in general can be grouped into four categories according to Tanaka and Kawamoto.^[8] The first one includes those methods which involve the binding of the biocatalyst to a water-insoluble support, by using ionic or covalent chemical links, bio specific coupling, or junctions due to adsorption phenomena. Natural polymers such as polysaccharides (cellulose, dextran, and agarose derivatives), proteins (gelatine and albumin), synthetic polymers (polystyrene derivatives and polyurethane) and inorganic material (sand, clay, ceramics, and magnetite) are commonly used for this purpose. A second category includes those methods using multifunctional compounds as glutaraldehyde, toluene or hexamethylene diisocyanate, to form Schiff's bases with functional groups in biocatalysts, thus producing water-insoluble agglomerate of catalysts.^[9]

The third category is constituted by those methods involving the trapping of the biocatalyst into a network formed by one or several polymers (polyacrylamide, alginate, carraginate, or synthetic resins), or those involving the embedding in membranes, encapsulating them inside microcapsules composed of synthetic polymers. A combination of the three former methods constitutes the last category. As we know, the adhesion of hydrophilic cells such as *Saccharomyces cerevisiae* is essentially dependent upon electrostatic interactions between the support and the normally negatively charged cell surface.^[10-13]

Materials and Methods

Laboratory scale 1 liter bioreactor (Remek make), Sodium alginate, Diammonium phosphate, sucrose, urea, cane molasses was collected from local area, Sodium chloride, calcium chloride was supplied from S D Fine chemicals, Technico Pvt ltd.

Methods

Fermentation process (batch culture process)

The required quantities of all the ingredients for the preparation of the medium with cane molasses, sugar, diammonium phosphate, pinch amount of urea were taken in the inoculation flask. Inoculated with 24hrs culture of *S.cerevisiae* seeded tank was kept under room temperature for a period of 24 hrs. After seeding process the total content was transferred to 350ml prepared and sterilized medium for two to three days. The inoculum tank was passed with high pressure steam through inlet and sent to outlet, making sure the inoculum tank become completely sterile. Upon completing the preliminary arrangements the total content was transferred to one litre lab scale fermentor (Remek make) for fermentation process. The duration of the bioconversion was optimized to 7 days. During this bioconversion period the total fermentation process was carefully monitored for pH, temperature, pressure and other parameters. The recovery of the ethanol from the fermentation broth was achieved by conventional double distillation process

Method of Immobilization

The commonly employed techniques for immobilization of enzymes are Adsorption, Entrapment, Cross-linking and Covalent bonding.

Gel entrapment: It involves entrapping of enzymes within the intestinal spaces of cross linked water insoluble polymer the usual method for formation is to polymerized the gelling agent in an aqueous solution of the soluble enzyme and then cross linking. While resulting get blocked can be mechanically dispersed into particles of desire size of the gel inert ably results in leakage of the entrapped enzyme even after the prolonged washing whilst the generation of free radicals in the reaction may be affect the activity of the entrapped enzyme.

A 2% sodium alginate gel was prepared by dispersion and mixing in the HCl buffer at Ph7 about 50ml. then lipase of varied quantities 100,200,300mg where added to the gel and there by mixed to make the distribution homogenous. The alginate gel containing lipase where then taken in a burette and the injected drop wise into 5% CaCl_2 of 50ml already prepared so that uniform size beads were formed. It was then kept for 20min and the beads where washed with double distilled water and with buffer.

Storage:

- The beads were dried and stored in a fridge.
- The whole reaction was carried out at 20-25⁰ c.
- Beads were separated and stored under 4⁰ c.

Yeast are eukaryotic micro organisms classified in the kingdom fungi with 1,500 species currently described estimated to be only 2% of all fungal species. Most reproduce asexually by mitosis. And many do so via asymmetric division process called budding. Yeast are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae as seen in most molds.^[9] Yeast size can vary greatly depending on the species, measuring 3-4µm in diameter although some yeasts can reach over 40µm. By fermentation the yeast species *Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohols for thousands of years the carbon dioxide has been used in baking and the alcohol in alcoholic beverages. It is also extremely important as a model organism in modern cell biology research and is one of the most thoroughly researched eukaryotic microorganisms. Researchers have used it to gather information about the biology of the eukaryotic cell and ultimately human biology. Other species of yeast such as *Candida albicans* are opportunistic pathogen and can cause infection in humans. Yeasts have recently been used to generate electricity in microbial fuel cells. And produce ethanol for the biofuel industry. Yeasts do not form a single taxonomic or phylogenetic grouping. The term yeast is often taken a synonym for *Saccharomyces cerevisiae*, but the phylogenetic diversity of yeast is shown by their placement in two spectra phyla, the ascomycota and basidiomycota. The budding are classified in the order Saccharomycetales.

Ethanol is one of the most advanced liquid fuels because it is environmental friendly. It is a clear colorless liquid with a characteristic agreeable odor. In dilute aqueous solution, it has a sweet flavor, but in more concentrated solutions it has a burning taste. It is an alcohol a group of chemical compounds whose molecules contain an OH group, bonded to a carbon atom. It melts at -114 °C, boils at 78.5 °C and has a density of 0.789 g/ml at 20 °C. ethanol is produced by fermentation when certain species of yeast metabolize sugar in the absence of oxygen, they produce ethanol and carbon dioxide. Ethanol is particularly useful in industrial applications because of its relatively high affinity for both water and organic compounds. The composition of other alcohols limits their flexibility as compared to ethanol. It is usually sold as industrial methylated spirits which is ethanol with small quantity of methanol added and possibly with some color. It is a bio fuel, which produced from biomass and wastes. Bio fuels provide an alternative to fossil fuel dependency and emit fewer pollutants.

Results and Discussion

The fermentation broth after the proper bioconversion period was taken aseptically for recovery process. The conventional recovery process of distillation is adopted for recovering the alcohol from the fermentation broth. Results based on utilization capacity of yeast *Saccharomyces cerevisiae* have been found under aseptic

conditions. The density and the alcohol content of free and immobilized yeast cells were found to be 0.9730, 0.9705 and 85%, 93.52% respectively. The optimized pH and temperature of free and immobilized yeast cells were found to be 5, 28-30°C and 7, 37°C respectively. The duration of bioconversion was accurately seven days and the quantity of yeast beads added were 0.1 gm/ 15 beads.

Conclusion

As the summary of the work the fermentation of optimization and the yield value of popular organic solvent (ethanol) was studied and reported. The specific gravity of the distilled alcohol by free and immobilized yeast cells were found to be 0.9730, 0.9705 at room temperature and the alcohol content was found to be 85%, 93.52% as per International Pharmacopeia respectively. The parameters were evaluated and compared for the conventional production of alcohol by immobilized yeast cells. From the above investigation it is clearly indicates that the immobilized yeast cells have produced more yield than conventional method.

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References

1. Prescott and Dunn's Industrial Microbiology, CBS Publishers and Distributors; 4th edition (1 December 2004), p 213-254
2. Industrial Microbiology by L.E. Casida, Published August 1st 1968 by John Wiley & Sons, p 165-193.
3. Miri Klein, Indra Neel Pulidindi, Nina Perkas, Aharon Gedanken, 2015. Heteropoly acid catalyzed hydrolysis of glycogen to glucose, *Biomass and Bioenergy* **76**: 61
4. Ge, X.M., Zhang, L., Bai, F.W. 2006. Impacts of yeast floc size distributions on their observed rates for substrate uptake and product formation, *Enzyme and Microbial Technology* **39**: 2-289
5. Ossain Zabed, Golam Faruq, Jaya Narayan Sahu, Mohd Sofian Azirun, Rosli Hashim, Amru Nasrulhaq Boyce 2014. Bioethanol Production from Fermentable Sugar Juice, *The Scientific World Journal* **14**: 1
6. Sang-Eun Lee, Ji-Eun Lee, Eun-Jin Kim, Joon-Ho Choi, Woon-Yong Choi, Do-Hyung Kang, Hyeon-Yong Lee, Kyung-Hwan Jung 2012. Immobilization of Yeast *Pichia stipitis* for Ethanol Production, *Journal of Life Science* **22**: 4-508

7. Tran, C.T.H., Kondyurin, A., Hirsh, S.L., McKenzie, D.R. and Bilek, M.M.M. 2012. Ion-implanted polytetrafluoroethylene enhances *Saccharomyces cerevisiae* biofilm formation for improved immobilization, *Journal of The Royal Society Interface* **9**: 76-2923
8. Tanaka, A., Kawamoto, T. 1999. Cell and enzyme immobilization. In: Demain AL, Davies JE, Atlas RM, Cohen G, Hershberger CL, Hu WS, Sherman DH, Wilson RC, Wu JH, editors. *Manual of industrial microbiology and biotechnology*. 2nd ed. Washington, DC: ASM Press. 94–102
9. Liang, L., Zhang, Y.P., Zhang, L., Zhu, M.J., Liang, S.Z., Huang, Y.N. 2008. Study of sugarcane pieces as yeast supports for ethanol production from sugarcane juice and molasses. *J Ind Microbiol Biotechnol* **35**: 1605–1613
8. Neerupudi Kishore Babu, Botcha Satyanarayana, Kesavapillai Balakrishnan, Tamanam Raghava Rao, Gudapaty Seshagiri Rao 2012. Study of Sugarcane Pieces as Yeast Supports for Ethanol Production from Sugarcane Juice and Molasses Using Newly Isolated Yeast from Toddy Sap, *Mycobiology* **40**: 1, 35
9. Päivi Ylittervo, Carl Johan Franzén, Mohammad, J. 2011. Taherzadeh, Ethanol production at elevated temperatures using encapsulation of yeast, *Journal of Biotechnology* **156**: 1-22
10. Ho Nam Chang, Nag-Jong Kim, Jongwon Kang, Chang Moon Jeong, Jin-dal-rae Choi, Qiang Fei, Byoung Jin Kim, Sunhoon Kwon, Sang Yup Lee, Jungbae Kim 2011. Multi-stage high cell continuous fermentation for high productivity and titer, *Bioprocess and Biosystems Engineering* **34**: 4-419
11. Gi-Wook Choi, Hyun-Woo Kang, Se-Kwon Moon, Bong-Woo Chung, 2010. Continuous Ethanol Production from Cassava Through Simultaneous Saccharification and Fermentation by Self-Flocculating Yeast *Saccharomyces Cerevisiae* CHFY0321, *Applied Biochemistry and Biotechnology* **160**: 5-1517
12. Chen, T.H., Wang, S.Y., Chen, K.N., Liu, J.R., Chen, M.J. 2009. Microbiological and chemical properties of kefir manufactured by entrapped microorganisms isolated from kefir grains, *Journal of Dairy Science* **92**: 7-3002
13. Ge, X.M., Zhao, X.Q., Bai, F.W. 2005. Online monitoring and characterization of flocculating yeast cell flocs during continuous ethanol fermentation, *Biotechnology and Bioengineering* **90**: 5

