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Production of Ethanol from Immobilized *Saccharomyces cerevisiae*

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ABSTRACT

Ethanol (also called ethyl alcohol, grain alcohol, drinking alcohol, or simply alcohol) is an organic chemical compound *S. cerevisiae* is the most employed yeast for ethanol production at the industrial level though ethanol is produced by an array of other yeasts, bacteria, and fungi. This paper reviews the current and nonmolecular trends in ethanol production using *S. cerevisiae*. Ethanol has been produced from a wide range of substrates such as molasses, starch-based substrate, sweet sorghum cane extract, lignocellulose, and other wastes The study was carried out on ethanol production from Immobilized Saccharomyces cerevisiae The immobilization was done with calcium chloride and sodium alginate the beads were formed. Fermentation was carried out for 7 to 8 days at 28°C then distillation was done and final ethanol produce was checked with an alcohol meter and ethanol produce was 13% from immobilized Saccharomyces cerevisiae. The process parameters optimized were substrate conc, pH, and urea conc. The values of the process parameters are 30% substrate conc, pH 4.5, and urea conc 0.5%.

Keywords- ethanol, immobilization, Saccharomyces cerevisiae, Sodium alginate, fermentation.

I. INTRODUCTION

As the rising need for ethanol is depicting a progressive increase over the past few years, there requirement is likely to fulfil high yielding production strains and economically viable process realization for the production of ethanol **(McCambridge et al., (2019).** One such process is yeast cell immobilization which assists faster fermentation rates by supplying higher cell densities per volume unit of fermentation.

There are additional technical and economic advantages to using immobilized cell fermentation compared to free cell systems, For example, high fermentation rate, high method of using and handling the substrate, long working period, reduce the cost required for inoculum development, facilitates their reuse and separation from the product to facilitate simple harvesting, increasing bioreactor productivity. The cost of bioprocessing by removing the long and costly process of cell recovery and cell recycling, reducing product inhibition, not reducing the desired biocatalytic activity of the cell, protecting against high shear damage, creating a favorable microclimate environment, declines the chances of contamination. And high alcohol tolerance **(Ingledew et al., 1999).** The beads formed by this process are completely active, flexible, and hard to withstand light movements **(Lee et al., 2011).**

Immobilization can be rendered as the imprisoned of all types of biocatalysts, including cellular organelles, enzymes, or cells, which can lead to reciprocation, however, are isolated from the majority of segments or the external environment. Immobilization includes a big selection of applications in several industries like biotechnology, pharmaceutical, environmental, food and biosensor industries**. (Yushkova et al., 2019)**

Various processes have been developed for ethanol production, but the worldwide demand for ethanol is usually met by biotechnological fermentation processes **(Nandy et al., (2018)** Many organisms, including Bacteria, Yeast, and Fungi have been tested for ethanol fermentation. The fermentation process of ethanol by these organisms, especially by immobilized yeast cells has been studied in a wide way. **(Khammee et al., 2021**). However, S. cerevisiae remains a favourite species, the same species used for making bread and for some wines or beers **(Umeh et al., 2016)** In pure and mixed cultures, S. cerevisiae presents almost equal yields and productivity. The appearance of the substrate greatly affects the ethanol fermentation process. Therefore, the raw material selected for ethanol fermentation is of great importance in the fermentation process **(Shih et al.,2009)** Hydrolysed enzymes are used to reduce complex sugars and then to ferment for high concentrations of ethanol. It is also made from various agricultural by-products such as cereals, fruit juices, fruit extracts, whey, sulphites waste alcohol and molasses **(Ul-Islam et al., 2020)** Molasses is obtained from a variety of sources such as sugarcane, beet and citrus**. (Álvarez-Cao, María-Efigenia, et al. (2019))** This is a syrup after removing the sugar from the mother syrup. Ethanol production is becoming increasingly important due to its wide range of applications, so researchers are constantly looking for alternative ways. Our main objective of this method is to extract ethanol with the help of as stable yeast cells as possible i.e., Saccharomyces cerevisiae. **(Larsen et al.,1994).**

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Morphology of Yeast

Fermentative yeast is a type of yeast that produces fermentable sugars. Saccharomyces cerevisiae is mostly employed in the manufacturing of ethanol. It is a suitable strain for the synthesis of ethanol substrate. It aids in the manufacture of bioethanol from sugar molasses. Because of its commercial availability and low cost, the S. cerevisiae bacterium was chosen. It has a wide range of uses in the food business. **(Asif et al., 2015)**

They are single-celled fungi. The size and shape vary by mutation and growth rate. It was found in *S. cerevisiae* during the time of the cell cycle its shape changes. Generally, they are larger than most of the bacteria; (1-5) um wide and (5-30) um length. **(Salari, R., & Salari, R. (2017)**. Yeast cell lacks flagella and another organ of locomotion. A cell wall composed of the thin chitinous cell wall. The protoplasm is surrounded by a cell membrane which contains all the usual cell organelles like ribosomes, mitochondria, ER, nucleus, and other granules. A vacuole is single, large and centrally located. Extracellular formation of sphingolipids, heteropolysaccharides, and phosphomimetic by yeast cells. A hair-like structure with a length of 0.5-7 nm was detected on the outer surface. Trehalose (0-16%), ribosomes, and glycogen are found in the ground cytoplasm (12 percent). a certain percentage of the nuclear membrane, which is made up of polyphosphate, DNA, and RNA and has pores of 85 manometers, is responsible for cell division and nuclear division. Yeasts generally reproduce by Asexual methods such as Budding or fission, Yeasts lack sex organs (antheridium and oogonium) **(Herskowitz, I. 1988).** Sexual reproduction in yeast is highly variable. **(Phale et al., 2018)**

Phosphomannans, -linked mannans, heteropolysaccharides, and sphingolipids are found on the outer surface of the capsule to display the fimbriae - hair-like structure with a diameter of 0.5-7 nm and a length of 0-10 m for flocculation. Polyphosphates, glycolytic and hydrolytic pentose cycle enzymes, glycogen (12%), trehalose (0-16%), and ribosomes are all found in the ground matrix, which is a fluid suspended in the cytoplasm. Ninety percent of the genetic material in a yeast cell is found in the nucleolus. Membranes are a pair of unit membranes with 85 nm pores that function to divide cells and nuclei**. (Sivignon, Adeline, et al. (2021))**

Saccharomyces cerevisiae that has been immobilized can also make ethanol. Immobilization of Saccharomyces cerevisiae is accomplished in a variety of methods. Immobilized cells are commonly utilized in continuous operations when the dilution rate exceeds the washout rate. In fermenters with free cells, the cellular concentration was higher, and the reactor capacity was smaller. The immobilization of yeast cells can be accomplished in a variety of ways, including the use of calcium alginate. Microorganisms consume glucose via the Embden-Meyerhof-Parnas route under anaerobic circumstances. **(Singh, Anita, et al 2013)**

II. RESEARCH METHODOLOGY

Material

Chemical Required

- Sodium hydroxide
- HCL
- Urea
- Glucose dinitro salicylic acid (DNS), potassium dichromate, sodium alginate, agar, sodium potassium tartarate, CaCl2, sulfuric acid, potassium dichromate was of analytical grades

Media Required

- YEPD Broth. Substrate Required
- Molasses.

Equipment Required

- Shaking Orbital Incubator
- Rotary shaking incubator
- Electronic Balance
- pH meter
- Spectrophotometer
- Heating mantle
- Autoclave
- Alcohol meter (gay Loussac temp 20°C and Range 0-100° by volume)

Miscellaneous Required

- Conical flasks
- Distilled water
- Syringe
- Beakers
- Micropipettes

Methods:

Maintenance of yeast:

The Baker's yeast maintained on (YEPD) broth containing yeast extract (1g/100ml), peptone (2g/100ml), glucose (2g/100ml) was obtained from the available stock culture. The flask was incubated at 28 °C for 2 days for maximum growth.

Development of inoculum:

100 millilitres of YEPD (containing yeast extract(1g/100ml), Peptone (2g/100ml), glucose (2g/100ml) was added in 500ml Erlenmeyer flasks. The flask was properly covered and autoclaved for 15 min at 121°C then allow to cool at room temperature, after cooling 2gm of yeast granules was added aseptically. Then the flask was kept in a shaking orbital incubator for 2 days at 28°C. (**Gut, Abraham Majak, et al., 2019)**

Method of Immobilization

Immobilization can be done with different methods here immobilization was done with the calcium alginate method **(Moreno-García, Jaime, et al 2018).** Firstly, 500ml YEPD broth was prepared (containing yeast extract) (5g/500ml)

Peptone (10g/500ml), glucose (10g/500ml) was added in 1000ml Erlenmeyer flasks then autoclave it for

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30 minutes then yeast and granules(10g/500ml) was added.

Put it for 4 days in a shaking incubator for growth. On the 5th-day immobilization process was done. Initially, we prepared 0.2M calcium chloride and let it chill in the refrigerator for 2 to 3 hours after the preparation of calcium chloride. We Prepared sodium alginate 3.5% growth medium then 30gm of the wet cell was added to the centrifuge tube. Took 10 centrifuge tubes with growth medium then centrifuge them for 15 minutes at 500rpm. After the centrifugation pellet was collected having 30gm weight mixed with a mixture of sodium alginate solution then chilled calcium chloride and syringe beads were formed.

Fermentation procedure

- 5 to 6 setup was taken then substrate conc, pH range, and urea conc were set.
- Add 10 ml of yeast inoculums and make a total volume of 200ml
- Kept them for 7 to 8 days in a shaking orbital incubator for fermentation at 28°C.
- After fermentation of 7 days samples were distilled.
- Measure the alcohol presentation with an alcohol meter.

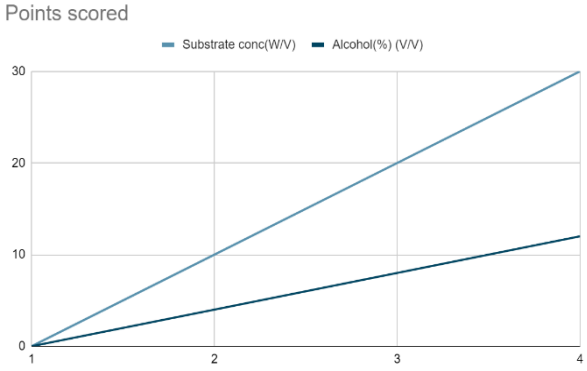
III. RESULT

Final ethanol production from immobilized saccharomyces cerevisiae…

The ethanol produced from immobilized yeast with the help of calcium chloride and sodium alginate by producing beads produces more ethanol comparatively with freely suspended saccharomyces cerevisiae. the parameter was sugarcane and the reasons were (30%,4.5, and .5) urea conc. the final ethanol produce was 13%.

Table 1.1 Final ethanol production from immobilized yeast

Serial no	Substrate conc (w/v) (%)	pH range	Urea (gm)	Inoculums (ml)	Final volume (ml)	Alcohol (%)
1	30	4.5	.5	10	200	13



IV. CONCLUSION

Finally, we conclude that ethanol produced from immobilized *Saccharomyces* was 13% and alcohol produced from different conc was measured by alcohol meter. All the parameter was optimized that was substrate conc, pH, and urea conc, and the optimized parameter was 30%,4.5, and .5%. After parameter optimization final ethanol produced.

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