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ISOLATION OF YEAST SPECIES AND THEIR EVALUATION FOR THE PRODUCTION OF BIOETHANOL BY FREE CELLS AND IMMOBILIZED CELLS

PRAJWAL V[1], RENUKA RAHUL[2], DR. NAIR SREECHA CHANDRAN[1]*

[1]M.Sc Biotechnology, St. George College of Management and Science, Bangalore, Karnataka, Mail id- prajwalgowdav23@gmail.com

[2]Senior Biology faculty, Daffodils international School, Pune, Maharashtra, Mail id- riturenurahul@gmail.com

[1*]Assistant Professor, Department of Biotechnology, The Oxford College of Engineering, Vishvesvaraya Technological University, Bangalore, Karnataka, Mail id- sreechanair@gmail.com

Corresponding Author (*)- sreechanair@gmail.com

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Abstract

The increasing demand for ethanol has led to research on alternative and unconventional raw materials. Bio-ethanol is a clean fuel for combustion engines and can reduce the environmental impact of fossil fuels. It can be successful by fermenting starches, sugar, ligno-cellulosic materials with ethylene. In 2019, global bio-ethanol production by the United States and the European Union. In the present study, YGP (Yeast Grape Juice), YSP (Yeast Sugarcane Juice) were used as sources for ethanol production. The study showed that ethanol produced by immobilized yeast cells had shown the higher ethanol concentration when compared to the ethanol produced from free yeast cells..

Keywords-: Bio-ethanol, YGP (Yeast Grape Juice), YSP (Yeast Sugarcane Juice), fermentation

1. Introduction

In recent times, the usage of gasohol in addition to its uses in sectors that need large-scale alcohol production has significantly boosted the demand for ethanol. The main energy source used worldwide is fossil fuel. A region's socioeconomic development.

Since its discovery over three centuries ago (Smith and Ferdinand, 1940), the manufacture of ethanol has grown in significance as a chemical feedstock for a variety of industrial solvents (Rose, 1967). It is employed as a fuel option, known as gasohol, in the current scenario with the fuel crisis (Wheals, *et al.,* 1990; Sybik Parker, 1999). Since the beginning of the automotive industry, ethanol has been utilized as an Otto engine fuel. The 1908 Ford T was the first commercial vehicle with the ability to run on gasoline, kerosene, or ethanol. The burning of fossil fuels contributes to climate change, global warming, and a number of energy and security issues. Fossil fuel is also not equitably distributed across countries and is not renewable. Since it can be produced from bioethanol, a liquid form of ethanol that is recognized to be a clean fuel for combustion engines, is an easily accessible alternative. The issues related to fossil fuels are lessened when bioethanol is used as an alternative, either as an octane enhancer or as the primary fuel. It is well known that fossil fuels produce greenhouse gas emissions that harm the environment. Burning fuels derived from petroleum raises the amount of CO_2 in the atmosphere, which directly contributes to global warming (Sybik Parker, 1999).

Research is being done on alternate and unconventional raw materials as a result of the rising demand for ethanol. The world's energy sector, especially that of Africa, is facing price increases and a critical need for greater security. Among the fuels that can be obtained from solid biomass is ethanol. In recent years, both the general public and the scientific community have paid close attention to biofuels. Oil, methanol, biodiesel, hydrogen and methane, often known as gaseous fuels, are some of the causes that have contributed to the increase in this newly discovered interest.

An essential industrial chemical, ethanol has the potential to replace fossil fuels with biofuels in the near future. Fermentation of sugars derived from plant waste can result in ethanol production. Irrespective of the substrate used, discussion and research analysis should be done regarding overall economics and energy usage (Demirbas, 2006). *Sacharomyces cerevisiae* is an organism which produces ethanol frequently therefore yeast strain is used as it has good specific growth rate and ability for ethanol production rate at high osmotic activity,

The ethanol concentrations necessitates a quick fermentation leading to increase in the production of high ethanol concentrations. There is a certainty for yeast growth to get decreased during batch fermentation process. Over the free cell method, microbial

immobilization shows technological and financial advantages. In order to reduce inhibition brought on by microbial concentrations, immobilization of microorganisms for fermentation are performed (Kourkoutas *et al.,* 2004). The main benefits of immobilizing yeast is an increase in ethanol yield and reduction in process costs. The purpose of this study was to analyse into the usage of sodium alginate for the batch ethanol fermentation of yeast cells.

2. MATERIALS AND METHODS

ISOLATION OF YEAST SPECIES

Grapes and sugarcane juice were collected from a local market located in Banaswadi (Bengaluru). Both the samples were kept for fermentation for 10 days in conical flasks. After fermentation, 1ml of each sample was followed by serial dilution. Plates were incubated at 30ºC for 3-5 days. Because yeast strains are typically exposed to comparable circumstances throughout the fermentative process, these particular conditions were chosen (2g/100mL dextrose) the medium was sterilized using an autoclave at 121°C, with 15 psi pressure. After the colonies had been stained with lactophenol blue, morphological appearances and a microscopic examination were performed and other test like yeast cell viability. (Dr. Ashish Shukla, May, 2020)

FERMENTATION OF SUBSTRATES BY IMMOBILIZED YEAST CELLS:

Preparation of immobilized beads:

Two colonies of yeast cells were selected that is, Immobilized Yeast Grape strain (YGP) and Immobilized Yeast Sugarcane strain (YSC) One loopful of culture was dissolved in sterile distilled water in an eppendorf tube and mixed into the Na-alginate solution. Transfer the suspension to the $CaCl_2$ solution in order to immobilize the cells. 8 suspensions of immobilized cells were prepared, four from each strain (YGP & YSC).

Substrate preparation: (Banana peel, Jagerry, Sweet potato & Molasses)

10gm of each substrate was weighed and crushed by using mortar and pestle. The crushed substrate was dissolved in 100ml warm distilled water. Then it was filtered by using a filter paper to remove the debris. The filtered substrates were sterilized by autoclaving at 121^0C, 15 psi pressure for 1 hour. After autoclaving, the substrates

were kept for cooling, then they were inoculated with 1ml of yeast suspension that is immobiloized Na-alginate beads in an aseptic condition in laminar air flow (LAF). And the inoculated substrates were kept for fermemtation for 4 days. (Marica Rakin, *et.al.*, November 2008)

Distillation of the fermented substrate of the immobilized yeast cells

Filter the fermented substrates using a filter paper. Then add the filtered substrate into the flask with a round bottom, attach the fractionating column to complete the assembly of the fractional distillation system. Connect the condenser to the fractioning column and place the distillate-capturing flask underneath to collect the distillate. Using the Heating mantle underneath the round-bottom flask, heat the mixture until it is above the boiling point of ethanol (80 degrees Celsius). Until the boiling stops, keep the mixture's temperature constant. At this moment, distillation is complete. (Pundlik Ware, et.al., May,2019)

PHYSICOCHEMICAL CHARACTERIZATION OF BIOETHANOL EXTRACTED:

The appearance of the bio-ethanol produced was observed colorless liquid by the naked eyes. (Er. Aarti Gautam, Dr. Ashish Shukla, May, 2020). The odour plays an important role, as a change in odour determines the progress of the fermentation process. As the fermentation progresses the odour turns to a strong alcoholic smell. (Er. Aarti Gautam, Dr. Ashish Shukla, May, 2020). 50ml of the sample was measured and added to a 100 ml beaker. The pH meter was turned on, and the testing electrode was inserted into the beaker of sample. The pH of the sample was calculated using the digital readout of the pH meter, which displayed the digits. (Er. Aarti Gautam, Dr. Ashish Shukla, May, 2020). It is common practice to measure the alcohol concentration of ethanol, wines, beers, distilled spirits, etc. Fruit juices of high grade almost never include alcohol. The other volatile components are responsible for the minuscule amount detected by the distillation process. An empty bottle was placed on weighing balance and the reading was recorded, the bottle was removed and filled with 50 ml of the distilled water which was later placed on a weighing balance, the reading was taken. The bottle was then removed and filled with 50ml distillated (extracted bioethanol). Calculate the specific gravity of the sample distillate and read

the alcohol percentage from table of specific gravity vs. alcohol % by volume. (Er. Aarti Gautam, Dr. Ashish Shukla, May, 2020)

ANALYSIS OF BIOETHANOL PRODUCED

A test tube was filled with around 5mL of distilled ethanol. It was filled with a pinch of $K_2Cr_2O_7$ and a few drops of H_2SO_4. The sample's colour changes from pink to green, indicating the presence of bio-ethanol (Thenmozhi and Victoria 2013) In order to determine the antibacterial activity, agar well-diffusion testing was created in 1940 (Heatley N.G., 1944). On the agar plates, *Escherichia coli* and *Bacillus subtilis* were swabbed. Wells were punched using a clean, sterile puncture. The wells were filled with the extracted ethanol samples in the range of concentration of 50µL, and the plates were then incubated at 37°C for 24-48 hours. The zone size (mm) was calculated following incubation. A clear zone denotes the bio-enzyme solution's antibacterial efficacy against microbes. (Er. Aarti Gautam, Dr. Ashish Shukla, May, 2020).

3. RESULTS AND DISCUSSION

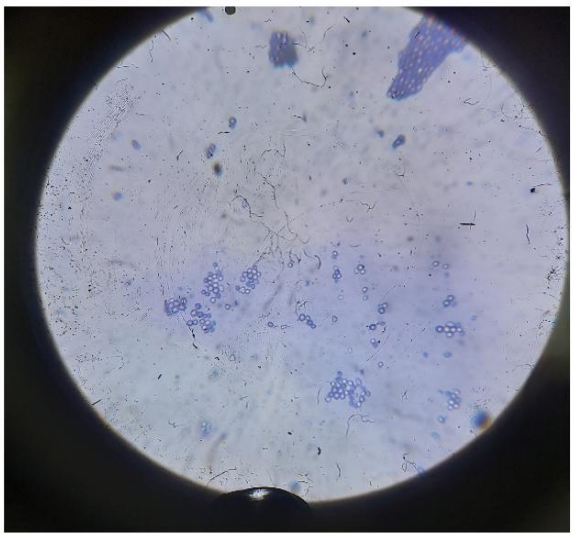
Isolation of yeast species

Many strains were isolated from grape and sugarcane juice source, two strains were selected one from each source through pre-fermentation by checking their ability to ferment the substrate and viability of the cells was more.

Identification of yeast species

Microscopic observation;

Ellipsoidal shape and budding yeasts were observed with specific division patter with 5-10µm in diameter**s.** Blue coloured, lactophenol cotton blue absorbed, yeast strains are seen under microscope **(Figures 1 and 2)**.



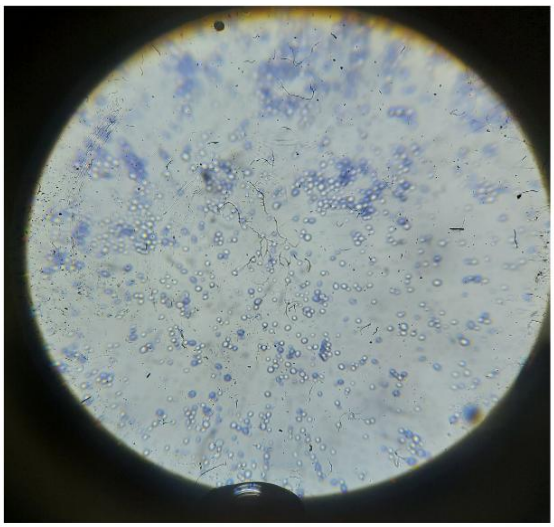


Figure 1: Lactophenol cotton blue staining staining(Grape strain)

Figure 2: Lactophenol cotton blue (Sugarcane juice)

Cell viability test

Cell viability test is done to observe the live cells before inoculating to the substrates, more colourless(transparent) live cells are observed by haemocytometer under microscope **(Figure 3)**. More colourless live (viable) cells were observed compared to dead (non-viable) cells in the ratio of 9:1.

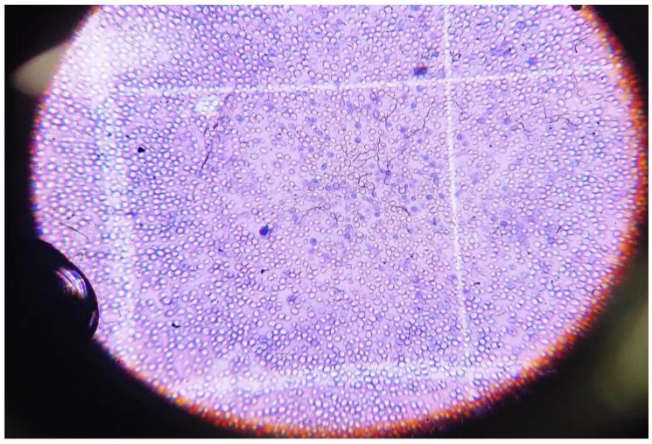


Figure 3: Yeast cell viability test

Flash point

Figure 4: Ethanol showing Flashpoint

The extracted ethanol produced flammable vapours that was ignited in the air by a flame above its surface. By heating the container to a particular temperature ethanol was tested for flash point **(Figure 4).**

Specific gravity

Specific gravity of the ethanol produced by free cells :

YGP (Yeast Grape Strain)

Table:1

Substrates	**W_3-W_1 (wt. of ethanol with SG bottle) gms**	**W_3 (wt. of ethanol) gms**	**Apparent SG= (W_3/W_2) gms**	**Percentage of alcohol (V/V)**
Banana peel	42.03-16.86	25.17	0.9988	0.79%
Jaggery	42.00-16.86	25.14	0.9976	1.60%
Sweet potato	41.91-16.86	25.05	0.9940	4.06%
Molasses	41.96-16.86	25.10	0.9960	2.69%

YSC (Yeast Sugarcane Strain)

Table:2

Substrates	**W_3-W_1 (wt. of ethanol with SG bottle-wt. of empty SG bottle) gms**	**W_3 (wt. of ethanol) gms**	**Apparent SG= (W_3/W_2) gms**	**Percentage of alcohol (V/V)**
Banana peel	42.04-16.86	25.18	0.9992	0.53%
Jaggery	42.03-16.86	25.17	0.9988	0.79%
Sweet potato	41.92-16.86	25.06	0.9944	3.79%
Molasses	41.97-16.86	25.11	0.9964	2.41%

Ethanol produced from free yeast grape strain by the substrate sweet potato had shown higher concentration of 4.06% relatively, banana peel, jaggery and molasses produced ethanol with lower concentration of 0.79%, 1.60% and 2.69% respectively.

Ethanol produced from free yeast sugarcane strain by the substrate sweet potato had shown higher concentration of 3.79% relatively, banana peel, jaggery and

molasses produced ethanol with lower concentration of 0.53%, 0.79% and 2.41% respectively (Table 1 and Table 2).

Specific gravity of the ethanol produced by immobilized cells:

YGP (Yeast Grape Strain)

Table:3

Substrates	**W_3-W_1(wt. of ethanol with SG bottle-wt of empty SG bottle) gms**	**W_3 (wt. of ethanol) gms**	**Apparent SG= (W3/W2) gms**	**Percentage of alcohol V/V)**
Banana peel	42.01-16.86	25.15	0.9980	1.33%
Jaggery	41.97-16.86	25.11	0.9964	2.41%
Sweet potato	41.89-16.86	25.03	0.9932	4.64%
Molasses	41.83-16.86	25.07	0.9948	3.52%

YSC (Yeast Sugarcane Strain)

Table:4

Substrates	**W3-W1(wt. of ethanol with SG bottle-wt. of empty SG bottle) gms**	**W3 (wt. of ethanol) gms**	**Apparent SG= (W_3-W_1/W_2-W_1) gms**	**Percentage of alcohol (V/V)**
Banana peel	42.02-16.86	25.16	0.9984	1.06%
Jaggery	42.01-16.86	25.15	0.9980	1.33%
Sweet potato	41.90-16.86	25.04	0.9936	4.36%
Molasses	42.00-16.86	25.14	0.9976	1.60%

4. Discussion

The socioeconomic development of any country has been greatly impacted by the rising demand for ethanol as a result of the extensive usage of gasohol in large-scale alcohol producing sectors. Fossil fuel is the primary energy source used worldwide, and the availability and consumption of energy have a big impact on the socioeconomic growth of a nation. With the help of living yeast cells, alcohol is produced from different substrates by fermentation processes, resulting in the production of ethanol, carbon dioxide, water, and heat by breaking down sugar molecules. Jaggery, sweet potatoes, molasses, and banana peels are the four different substrates that are utilized to make alcohol. In the present investigation, different

yeast species were isolated from two distinct sources, namely; grapes and sugarcane juice. The alcohol producing capabilities were assessed by using free cells and immobilised cells. Further comparative studies were carried out in order to know the percentage of the alcohol produced (Thenmozhi and Victoria 2013).

The present study included the isolation of yeast species from grape and sugarcane juice sources, two strains selected through pre-fermentation. The yeasts were observed under a microscope and showed an ellipsoidal shape with specific division patterns. Yeasts were stained with lactophenol cotton blue staining and determined the cell viability. Ethanol produced from immobilized yeast grape strain by the substrate sweet potato had shown higher concentration of 4.64% relatively, banana peel, jaggery and molasses produced ethanol with lower concentration of 1.33%, 2.41% and 3.52% respectively. Ethanol produced from immobilized yeast sugarcane strain by the substrate sweet potato had shown higher concentration of 4.36% relatively, banana peel, jaggery and molasses produced ethanol with lower concentration of 1.06%, 1.33% and 1.60% respectively (Table 3 and Table 4). According to the values obtained from the AOAC (Association Of Analytical Chemistry) chart, the ethanol produced by immobilized yeast cells had shown the higher ethanol concentration when compared to the ethanol produced from free yeast cells.

5. Conclusion

The study found that the ethanol produced from immobilized yeast cells showed apparent results to that of the ethanol produced from free yeast cells. Additionally, ethanol produced from immobilized yeast cells showed greater zone of inhibition with reference to ethanol produced by free cells. Though immobilized cells produced the ethanol with higher concentration, sometimes there may be a possibility of loss of biological activity of the cell. Immobilization is moreover an expensive technique and often require sophisticated equipment. Beside all these circumstances immobilized yeast cells had an extreme potential to convert sugars into ethanol in contrast to free yeast cells.

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