Helix (2022) 12 (3): 28-33

https://doi.org/10.29042/2022-12-3-28-33

Study on the influence of Abiotic Stress on Growth and Alcohol production by Yeast

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Received: 18th April 2022, Accepted: 20th May 2022, Published: 30th June 2022

Abstract

Use of microbes as bioreactors for the industrial production of several metabolites and products of commercial importance have been one of the prominent applications in science and technology. Especially industrial scale production of alcohol using Yeast *Saccharomyces cerevicie* has been the practice since long and yet most demanded. In view of the commercial importance of alcohol production by yeast, the current work aimed to analyze several inoculation and incubation conditions like, temperature, pH, Chemicals etc on the growth and production of alcohol by baker's yeast. The yeast required is obtained from a grocery store which acts as a seed culture for the production of mother plate followed by its use in the inoculation of alcohol production media. The effect of various growth conditions on the growth and alcohol production were studied using spectrophotometry. Based on the statistics the optimal conditions for high growth and good enzyme production were calibrated.

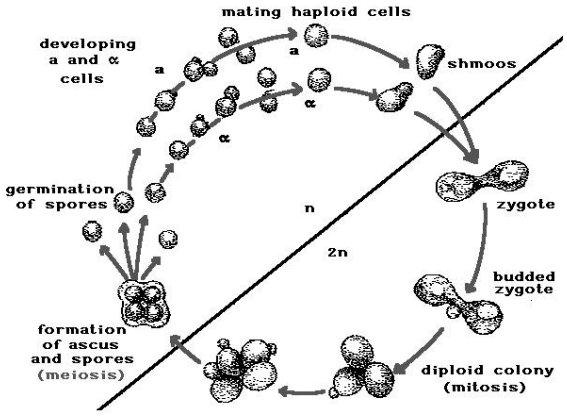
Key words: Optimal conditions, Bioreactors, Metabolites, Spectrophotometry, *Saccharomyces cerevicie*

Introduction

Yeast, Saccharomyces is known to be the model organism for all the genetic and evolutionary studies related eukaryotes. Apart from its importance in research and development it is known to posses a high commercial value. In nature yeasts are widespread and one of the most common habitat for these organisms is grapes [3] skin exterior and the inner intestinal tract of warm blooded animals. These minute single celled organisms show the complexity of cell very much similar to the higher multicellular organisms making it a model for study [2]. They are very fast replicating with a time of division being very less i.e 90 minutes in contrast to bacteria and other organisms [8].

S. cerevisiae, the baker's yeast was first subjected for whole genome sequencing in the year 1996 which revolutionized the genetic research [4] . Budding is the basic mode of replication performed by Yeast for its division. Each progeny cell is obtained as a small bud like projection on the parent cell which detaches and develops into a whole new organism. Cell cycle of yeast is composed of 4 major stages which include G1, S, G2 and M phase [13] and these phases are regulated identical to the regulation seen in eukaryotes. The cell continues to divide asexually under normal nutrition available conditions. Figure 1 shows the life cycle of yeast.

Fig1 : Budding yeast life cycle



Picture Credit: *Wikicommons [10]*

Alcohol production by yeast:
Yeast has long been considered for its importance in the production of alcoholic beverages, bread, carbonated dough for bakery products and a large variety of industrial products [14, 6]. These organisms are known to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic drinks like beer and wine. Brewing yeasts are of 2 major varieties, top-fermenting yeast and bottom-fermenting lager yeast. *S. cerevisiae* belongs to the top fermenting group which raises to the surface during fermentation and are used to brew ales, stouts, wheat beers etc [12].

Materials and Methods

Pure yeast culture preparations [9]:
Yeast can be kept on solid agar plates, or grown in liquid culture. Solid agar plates - 9cm diameter plastic Petri dishes filled with solidified media. Plastic disposable loops are used to streak yeast on agar plates, they are then inverted and put in an incubator at 25^0 or 30^0C until colonies ~1mm across have formed. (Suspend 10 g agar in 1 litre of distilled water. Heat to dissolve. Add 0.5 g K2HPO4 , 0.2g MgSO4.7H2O, 0.2 g NaCl, 0.2 g, 10 g mannitol and 0.4 g yeast extract. Dispense as required and sterilize.)

Pure broth culture preparation:
YEMA broth was prepared by adding 1 litre of distilled water 0.5 g K2HPO4 , 0.2g MgSO4.7H2O, 0.2 g NaCl, 0.2 g , 10 g mannitol and 0.4 g yeast extract. The broth is heated to dissolve the components. Dispensed as required and sterilized in an autoclave [11]. Sterilized and cooled broth was inoculated with the seed culture to obtain the pure liquid suspension of yeast.

Studying the effect of various conditions of Temperature, pH and UV irradiation on the growth and metabolism of yeast.
The general conditions which would influence the active growth and metabolism of yeast include pH which plays an essential role in enzyme activities and chemical reactions, Temperature that can have a vital influence on functionality of enzymes and proteins, and some chemicals. Various parameters selected for the study include:
Temperatures: 20, 37, 40, 60
pH: 3, 5, 7, 9
UV irradiation: 10sec, 20 sec, 30 sec, 40sec

Mannitol salt broth was prepared and dispensed in 13 different labeled test tubes and subjected for sterilization [7]. All the tubes were labeled and regulated according to the test criteria pH, Temperature and UV exposure. In case of UV exposure the inoculated tubes were kept under UV light in a Laminar airflow for the regulated time interval. All tubes were incubated at 37^0C overnight except the tubes labeled for temperature. These tubes were incubated according to their prescribed test temperate.

Turbidometric estimation of yeast growth [15]:
After the incubation of 48hrs of previously inoculated test samples with yeast the samples are subjected for spectrophotometric estimation of turbidity which imparts to the measure of the growth rate of yeasts in the provided media and incubated conditions. All the tubes were brought to the working table and 1.5 ml of each broth was collected into the cuvette and OD was measured at 600nm.

Production of alcohol using the above treated culture:
Alcohol fermentation media was prepared as per the composition and sterilized [5]. The media was dispensed in 13 labelled test tubes which includes one to be control. All the tubes were inoculated with the respective samples as prepared in the previous step. The media was incubated in a shaker flask incubated to provide good aeration for a period of 4 days which was followed by downstream processing to collect alcohol thus produced.

Alcohol production media:
YPD is the traditional media for this purpose that contains 10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose/glucose into distilled water. Incubation needs shaker flask set at 37^0C. For the samples which involves the study of optimum temperature incubation is done according to their pre designed values. After the prescribed incubation time of 4 days the broth was subjected for centrifugation and the supernatant containing alcohol was separated. The supernatant was than subjected for alcohol estimation.

Alcohol estimation :
Alcohol produced in the above step is called “wash”, and is about 15 to 18% pure. In order to obtain pure ethanol factional distillation must be performed. In the current work the crude supernatant was subjected for alcohol content estimation to check for the effect of various factors on alcohol production.

The current study uses a redox titration method for estimating the amount of alcohol produced in the broth. The method was based on Conway, 1939 [1]

Ethanol produced in the medium is converted to acid using excess of Acid dichromate under acidic condition. This coverts the ethanol to ethanoic acid. The unreacted acid dichromate is estimated to calculate the alcohol produced. The above solution is subjected for titration against sodium thiosulphate solution. Acid iodide and starch is used as indicator system. In this procedure addition of acid dichromate (yellow color) to the test sample yields a brown color solution. To this acid iodide is added. This is titrated against the base sodium thio sulphate, which re imparts the solution its yellow color due to the formation of Iodine. At this stage 1% starch is added as an indicator that imparts the solution a typical blue color. Upon further titration with sodium thiosulphate an end point is reached indicated by discoloration of solution. Control without test sample is also included in the titrations.

The amount of ethanol produced can be calculated as shown below:
volume of the sodium thiosulfate solution used for the sample titration - volume used for the blank titration

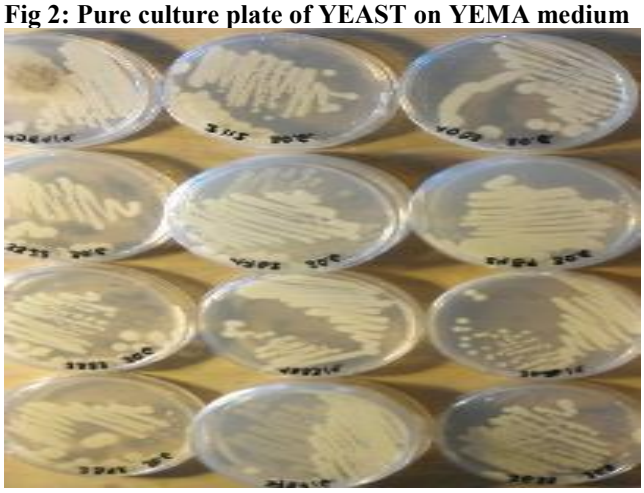
This volume of the sodium thiosulfate solution is now used to determine the alcohol concentration. Number of moles of sodium thiosulfate can be calculated in this volume. Using the equations, determine the relationship between the moles of sodium thiosulfate and the moles of ethanol. 6M of thiosulphate is equivalent to 1M of dichromate and 2M of dichromate is equivalent to 3M of ethanol
Therefore : 1Mof thiosulphate is equivalent to 0.25M of Ethanol
This ratio can be used to calculate the moles of alcohol in the sample solution.

$Cr_2 O_7^{2-} + 14 H^+ + 6 I^- \rightarrow 2 Cr^{3+} + 3 I2 + 7 H_2 O$

Results and Discussion

Initial part of the work included the seed culturing of the Yeast for activation and to obtain homogeneous cell suspension with increased cells. The plates showing isolated colonies and the liquid suspensions were made. All the liquid suspensions made as 13 samples were treated differently and inoculated for the production of alcohol.
Turbidity was used as a measure of growth rate and alcohol produced was measured based on Redox Titration method. All he results are furnished hereunder.

Fig 2: Pure culture plate of YEAST on YEMA medium



All the plates were than used as seed cultures for inoculation into different labeled production medium in tubes. This included the estimation of OD for different time intervals whose results are noted further.

Fig 3: Collection of supernatant for alcoho estimation

Figure 3 shows the tubes containing supernatant obtained from production media which will be subjected for alcohol estimation.

The effect of various Temperatures, pH and time of UV exposure on both growth and ethanol production by Yeast were studied under lab conditions and the following conclusion were derived.

Table 1: Estimation of Growth rate of Yeast based on Turbidometry using Spectrophotometer:

Samples	Values of parameters	OD Value at 600nm After respective incubation times				
		10hrs	14hrs	18hrs	24 hrs	28 hrs
T1	20	1.7	3.1	5.3	5.1	4.0
T2	37	1.7	3.3`	4.8	4.8	4.5
T3	40	1.8	3.2	5.6	5.9	4.8
T4	60	0.8	2.7	3.5	3.7	3.0
P1	3	0.5	1.3	2.7	2.5	2.0
P2	5	1.8	3.1	4.1	4.3	3.8
P3	7	1.6	3.6	4.3	4.5	3.2
P4	9	1.5	3.2	3.9	4.0	3.5
U1	10	1.2	2.4	2.8	2.9	2.6
U2	20	0.9	1.9	2.4	2.5	2.5
U3	30	0.5	1.2	2.8	2.6	2.3
U4	40	0.2	0.8	1.2	1.1	0.9

From the above table showing the turbidity of the media at regular time intervals, it can be observed that the values initially increase indicating an exponential phase followed by a lag phase where the bacteria stop further division. There is also a decline phase observed by a decrease in the OD values indicating the death of cells at the end of growth phase. Highest growth was observed in the tubes maintained at 40^0C, 37^0C temperature and pH of 7.

Table 2: The effect of various conditions on the production of alcohol.

Tube	Volume of Sample in ml	Volume of Acid Dichromate in ml	Volume of Dist water in ml	Volume of KI in ml	Volume of starch in ml	Ethanol produced in gms per Litre based on thiosulphate used
Blank	0 (water 1ml)	10	10	1	1	0.00
T1 (20)	1	10	10	1	1	27.6
T2 (37)	1	10	10	1	1	43.7
T3 (40)	1	10	10	1	1	46.4
T4 (60)	1	10	10	1	1	5.8
P1 (3)	1	10	10	1	1	4.6
P2 (5)	1	10	10	1	1	23.6
P3 (7)	1	10	10	1	1	40.6
P4 (9)	1	10	10	1	1	22.5
U1 (10)	1	10	10	1	1	22.4
U2 (20)	1	10	10	1	1	20.4
U3 (30)	1	10	10	1	1	11.5
U4 (40)	1	10	10	1	1	5.9

The amount of ethanol produced in grams per litre was calculated and the data is furnished above in the table.

Based on the Redox Titration study the optimum conditions required for the maximum production of ethanol were: Temperate of 40^0C, pH of 7. It was also observed that the exposure to UV irradiation adversely effects alcohol production. Thus UV exposure time and ethanol production are inversely proportional to each other.

https://www.canterbury.ac.nz/media/documents/science-outreach/ethanol.pdf

Conclusion

Ability of the YEAST to convert sugars into alcohol has been used in the study for the lab scale production of ethanol. Further the effect of various parameters like Temperature, pH, Exposure to UV on both growth and metabolism of the yeast cells was analyzed. The treated cells were used for the production of alcohol followed by its recovery. The alcohol thus produced was estimated using the method of Redox titration which used Starch ad KI as indicators. The total amount of ethanol produced under various conditions was estimated. The results show that the highest growth was observed for the pH 7, temperature 40 and no UV exposure. The optimum conditions required for the growth and production of alcohol by yeast were identified to be 40^0C temperature and pH of 7. Further the mutational effect of UV irradiation adversely effects the alcohol production.

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