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Production and optimization of ethanol production from spent tea waste using Saccharomyces cerevisiae

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ABSTRACT

Tea is an aromatic beverage prepared from the leaves of camellia sinesis. It is the most widely consumed drink in the world next to the water. Extracts of tea leaves contains polyphenols, amino acids, vitamins, polysaccharides and has a stimulatory effect due to its caffeine content. Consumption of tea in India remains modest and due to the presence of carbohydrates tea waste spent is used as a potential substrate for ethanol production. However worldwide demand for ethanol production has been increased in recent years so utilization of left-over tea spent for ethanol production may reduce the cost of fermentation process. optimization conditions for high levels of ethanol production from left over tea spent using saccharomyces cerevisiae were determined by alterations of temperature and pH and finally comparative studies of ethanol production using natural tea spent media to that of commercial production medium leads to the conclusion that the activity of tea spent in ethanol production was slightly high to that of commercial production medium.

Keywords— *Tea spent, Ethanol, Camellia sinesis, Fermentation, Production media, Saccharomyces cerevisiae*

1. INTRODUCTION

Tea is an affordable, inexpensive, aromatic beverage which is addictive due to the presence of methyl xanthine, theobromine and theophylline which act as a stimulant (Macfarlane et al., 2004). It is commonly prepared from the leaves of the evergreen shrub *Camellia sinesis* whose leaves and buds are used for tea processing belongs to the family Theaceae. Extracts of tea contains high amounts of polyphenols(30-40%) (Harbowy ME., (1997)), Amino acids(6%), carbohydrates(11%), enzymes in traces and 28 mineral elements which lends to the sweetness, good aroma, rich antioxidants and a sense of relaxation upon consumption. However based on the tenderness of the leaves different collections of tea are available for human consumption like Black, Dark, Oolong, yellow, Green and white tea .

India is the world's largest tea drinking nation with a consumption of 750gms per person per annum (Sanyal S., 2008). But this is slightly increased to 800gms in 2020 according to statista market forecast. However worldwide demand for ethanol production has also been reached to great heights because of its wide applications as a conventional fuels, Industrial, food purpose and even in hospitals as a sanitizers (Chiaramonti D et al., 2011; Govumoni et al., 2013).Hence the utilization of waste tea spent as a raw material for ethanol production is highly encourage able. According to statista market forecast at present, the consumption volume of tea in India for the fiscal year 2019 is approximately one billion kilograms. This number indicates the utilization of waste tea spent as the most prominent source for ethanol production by Saccharomyces cerevisiae.

Saccharomyces cerevisiae is a unicellular yeast measures about 3-4micrometres in diameter is the most commonly used strain for ethanol production and vegetative cell appears gram positive in nature. Other strains that can be used are *S.carlsbergensis, S.diasensis, S.exiquis, S.kluyveri, S.bayanus* (Jeff cox., 1999, D .Bird., 2005, Fugelsang K et al.,2010). The present work deals with the utilization of waste tea spent for the ethanol production along with the optimization studies using factors like temperature and pH which are essential for the maximum ethanol yield and low production cost of ethanol. A comparative study between the natural production medium (waste tea spent) with the Commercial production medium for alcohol production will also be discussed in detail in the present work.

2. METHODOLOGY

2.1 Preparation of Inoculum

2 gms of Baker's yeast was added to 100ml flask that contains 25ml sterile distilled water and incubated for 1-2hrs for the activation of species. A loopful of inoculum was streaked on YMPDA agar plates prepared as per the composition listed in table 1. The plates were incubated at 37°C for 24 hrs. Pure cultures were maintained on YMPDA slants and stored at low temperatures for further use.

Yeast cells were collected from the YMPDA plates and was inoculated in 200ml of sterile YMPD broth and incubated for 12 hrs at 37°C.Cells were collected by filtration and were suspended in sterile distilled water so that 3% inoculum should be maintained. (Santos et al., 2012).

Table 1: Composition of YMPD AGAR

S.no	Ingredients	Gms/litre
1	Yeast extract	4
2	Malt extract	3
3	peptone	3
4	dextrose	20
5	agar	20
6	Distilled water	1000ml

2.2 Preparation of production media

Three tea samples, Basil tea (Tulsi tea), Tea dust (three roses) and Green Tea were used for ethanol production. The waste tea spent of the three samples was air dried and powdered. 5gms of powdered tea was weighed and was added to 250ml of sterile distilled water, pre boiled for 10 min and autoclave at 15lbs /inch^2 pressure for 20 min. Similarly, the process was repeated with the other tea samples.

2.3 Fermentation process

To the sterile production medium (waste tea spent) add 0.5 ml of inoculum and incubate the flasks without agitation at 37°C for 48-72 hrs and the alcohol liberated was measured using dichromate method.

2.4 Estimation of alcohol by dichromate method

Prepare a standard alcohol by taking different aliquots of 2% alcohol and make up the volume to 5ml using distilled water. Then add 1ml of $K_2Cr_2O_7$ followed by 5ml of Conc.H_2SO_4 to all the tubes and the optical density was measured at 610nm. (Johnson. W.A et al. ,1999)

2.5 Optimization studies

To one set of Waste tea spent medium (50ml each), 200µl of yeast was added and incubated at different temperatures of 4°C, 25°C, 37°C, 50°C and 70°C for 3 days. Similarly, to other set of production medium pH of 2,4,6,8 and 10 was maintained and incubated at 37°C for 72hrs and the liberated alcohol was measured using dichromate method.

2.6 Comparative studies

As per the composition listed below in table 2 and 3, 200 ml of natural production medium and commercial medium were prepared and inoculated individually with 200µl of yeast and incubated at 50°C temperature with pH 8 for 48-72hrs and the liberated alcohol was measured

Table 2: Composition of Natural production medium

S.no	Ingredients	Gms/litre
1	Powdered Waste Tea spent	25
2	Distilled water	1000ml

Table 3: Composition of Commercial production medium

S.no	Ingredients	Gms/litre
1	Peptone	3
2	Sucrose	10
3	Yeast extract	3
4	KH_2PO_4	1
5	$(NH_4)_2SO_4$	5
6	NaCl	5
7	$MgSO_4.7H_2O$	0.5
8	Distilled water	1000ml

3. RESULTS

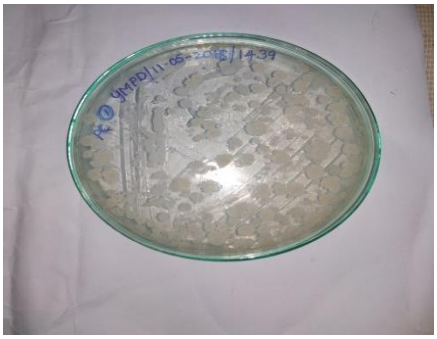


Fig. 1: Colony morphology

Yeast cells grew as a white colour round shaped colonies on YMPD Agar medium and round shaped cells were observed under microscope as shown in figure 2.

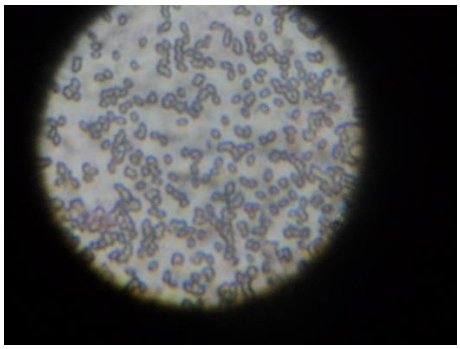
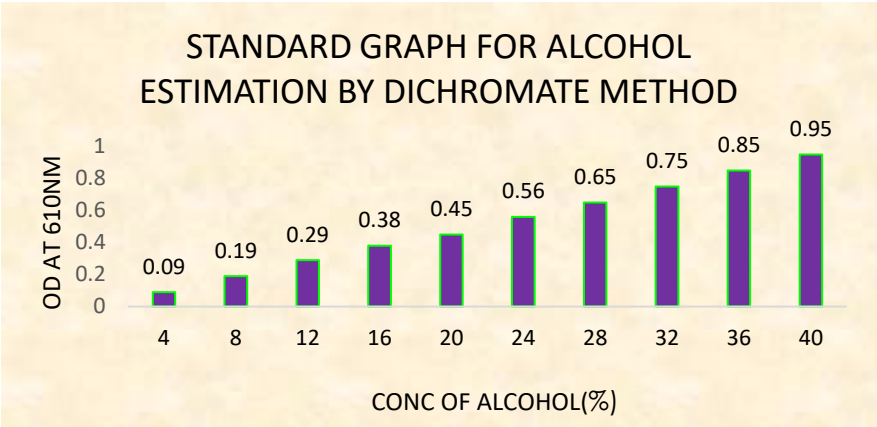
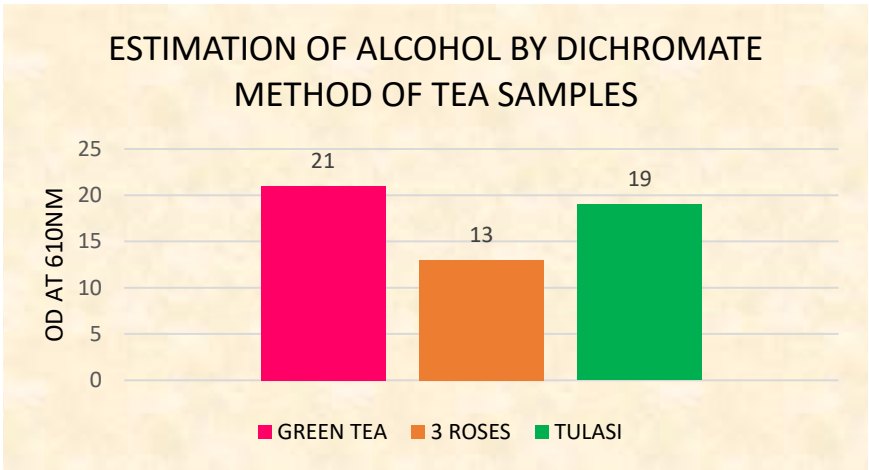


Fig 2: Yeast cells under microscope

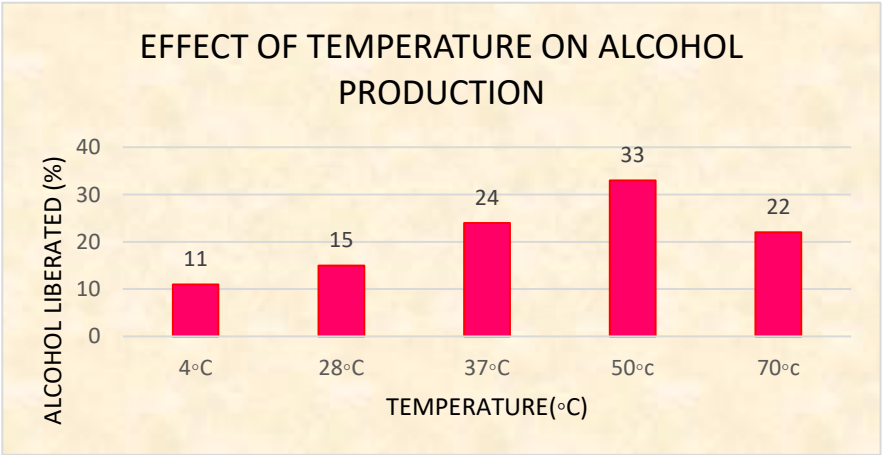
3.1: Standard graph for estimation of alcohol by dichromate method



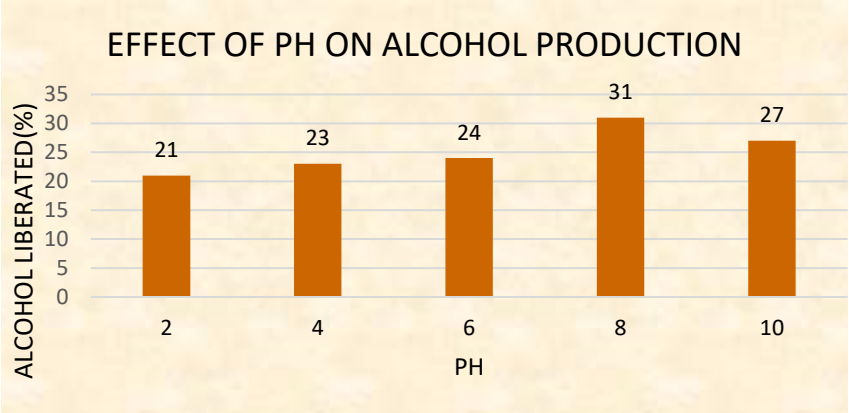
3.2: Estimation of alcohol from tea samples using dichromate method



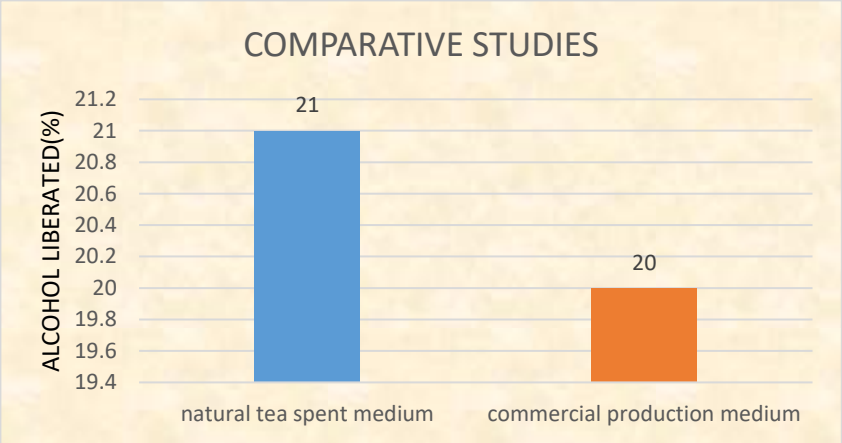
3.3: Effect of temperature on alcohol production (In green tea)



3.4: Effect of pH on alcohol production (In green tea)



3.5: Comparative studies between natural and commercial production medium



4. DISCUSSION

Among the three tested tea samples -Basil tea, three roses, green tea, the alcohol production was high in green tea with 21% followed by basil tea with 19% alcohol production. However, Yucel and Goycincick in 2015 has reported that the ethanol production was 12.72g/litre with black tea spent. Compared to their study the present study had shown high amounts of alcohol produced from green tea spent (21%).

Hence optimization studies were performed by using green tea waste spent which would be a good source for ethanol production. Temperature had showed its marked influence on the alcohol production by *Saccharomyces cerevisiae*. Maximum enhancement of alcohol production was seen at temperature 50˚c with 33% alcohol followed by 37˚c with 24% of alcohol. However, organism showed its minimum activity at 4˚c with only 11% liberation.

pH plays an important role in the enzyme catalysis next to temperature. Yeast cell had shown its maximum activity at pH 8 with the liberation of 31% minimum at pH 2 with 21% alcohol liberation. However, yeast didn't show much effect on pH variations as it produces almost the same with slight variations.

Comparative studies had found that at temperature 80◦c and pH 8 *Saccharomyces cerevisiae* produced slightly high levels of ethanol using waste tea spent as a raw material with 21% than commercial medium with 20%. Comparative studies proved that almost equivalent amount of ethanol production was produced. On comparison one can say that waste tea spent medium can be used as an alternative for commercial production medium for the production of ethanol industrially on small scale level.

5. CONCLUSION

Of all the tested tea samples, green tea spent waste showed maximum ethanol production by using *Saccharomyces cerevisiae* at a temperature of 80˚c and pH 8. The activity was almost equivalent to that of commercial production medium. From the above results, we conclude that the leftover tea spent can be used as an excellent and inexpensive raw material as it can reduce the economy of industrial production of alcohol.

6. ACKNOWLEDGEMENTS

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