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PRODUCTION AND OPTIMIZATION OF ETHANOL FROM FRUIT AND VEGETABLE WASTE USING SACCHAROMYCES CEREVISIAE

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Abstract

Industrialization and rapid population growth has increased the consumption of crude oils, coal and natural gas from time to time. In order to reach the demands of the people, conventional methods of biofuel production is highly essential. Most commonly used biofuel is ethanol which is primarily produced by sugar and starchy materials followed by lignocellulosic materials. This Paper reviews that the waste material of fruits and vegetables can be used as an excellent source of raw material for ethanol production using Saccharomyces cerevisiae by which we can reduce the economy of ethanol production industrially. However, optimization studies using different physical factors may also enhance the level of ethanol production. And finally comparative studies between the fruit and vegetable waste with that of synthetic commercial production medium leads to the conclusion that the highest alcohol yield could be produced using fruit and vegetable waste than that of commercial medium.

Keywords: Fruit waste, vegetable waste, Biofuel, Ethanol, Industrialization

1.0:Introduction

Bioethanol is an alcohol made by fermentation process obtained from carbohydrates, cellulosic biomass (Cadoche and Lopez ,1989; Bjerre et al. 1996)and fats. It can be used as an energy driven force in automobile industry as well as a gasoline additive to improve vehicle emissions. According to Department of energy (DOE) total consumption of alcohol as gasoline in USA is approximately 140 billion gallons. Apart from this India became a leading producer by 2020 with total ethanol production capacity of 223.87 Crores lit/ per annum. Rise in urbanization and Industrialization leads to the increase in the consumption of fossil fuels like coal, crude oils and natural gas, which leads to scarcity of these resources. As a result, people use alternative energy sources like biofuels, which is inexpensive and easily could produce by microbial actions. Bioethanol has equal importance like fossil fuels that act as a conventional fuel also has wide applications in industrial, food purpose and even in hospitals as sanitizers (Chiaramonti D et al., 2011; Govumoni et al., 2013). This could eventually leads to the huge demand in the global market. Therefore, the utilization of waste fruit and vegetables as a raw material for ethanol production is highly encourageable, which may lead to reduce the economy of industrial production of ethanol. Fruit waste and vegetable waste are the rich sources of carbohydrates, proteins, fats, minerals, fibres and fruit peel contains more amount of polyphenols. According to statista forecast global fruit production has reached to 868.1 million metric tonnes in 2018 while vegetables has reached 182 million metric tonnes in 2017. An estimation of 1.3 billion tonnes of

food is wasted globally (FAO Reports). 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, Fugelsang K et al.,2010). *Saccharomyces cerevisiae* is used as a model organism of study in industrial and research applications (Legras, et al., 2007).Global fruit production has experienced a remarkable increase. As a result, the left over waste food results in the emission of 8% human made greenhouse gases (Bos and Hamelinck, 2014). The present work deals with the utilization of fruit waste and vegetable waste 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. Comparative studies between the natural production medium (waste fruit and vegetables) with the Commercial production medium for alcohol production will also be discussed in detail in the present work.

2.0 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 1-2 hrs 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 medium:

2.2.1: Production medium (A)

500gms of weighed fruit waste that includes both peel and pulp of spoiled mango, grapes, banana, papaya, musk melon and pomegranate was taken and mashed using sterile water and autoclaved the medium at 15 lbs/inch^2 pressure for 20 minutes .

2.2.1: Production medium (B)

500gms of weighed spoiled vegetables like potato, tomato, brinjal, bottle guard from kitchen was collected and mashed using sterile water and autoclaved the medium at 15 lbs/inch^2 pressure for 20 minutes

2.3: Fermentation process:

To the sterile production media(A and B)individually add 1 ml of inoculum and incubate the flasks without agitation at 37°c for 48-72 hrs and the alcohol liberated was measured by using the below methods.

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:

2.5.1: Effect of incubation period

To one set of production medium (A) 50ml each,200µl of yeast was added and temperature of 37°C was maintained and incubated by varying the period of 36hrs, 72hrs,108hrs and 144 hrs. Similarly, the process was repeated with the production medium (B) and the liberated alcohol was measured using dichromate method.

2.5.2: Effect of temperature

To one set of production medium (A) 50ml each,200µl of yeast was added and incubated at different temperatures of 20°C, 37°C, 50°C and 80°C for 3 days. Similarly, the process was repeated with the production medium (B) and the liberated alcohol was measured using dichromate method.

2.5.3: Effect of pH

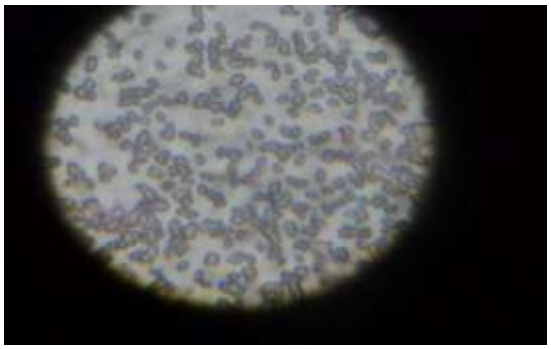
To one set of production medium (A) 50ml each,200µl of yeast was added and different pH of 5, 6, 7.4,8.7 was maintained and incubated at 37°Cfor 3 days. Similarly, the process was repeated with the production medium (B) and the liberated alcohol was measured using dichromate method.

2.6 Comparative studies:

As per the composition listed below in table 2, 500 ml of commercial medium and 500 ml of natural production medium (A) and (B) were prepared and inoculated individually with 500µl of yeast and incubated at 37°C temperature with pH 5 for 72hrs and the liberated alcohol was measured using dichromate method

Table 2: 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



3.0: Results

Fig 1: yeast cells under microscope

Round shaped yeast cells were observed under microscope as shown in figure 1

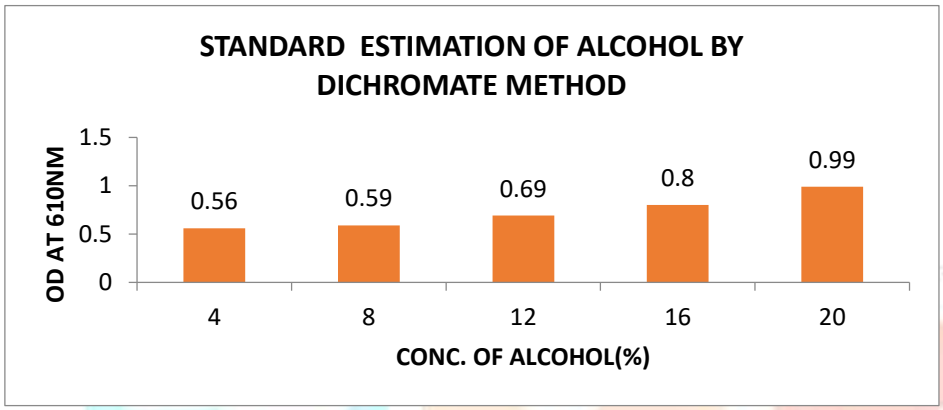


Fig 3.1: Standard graph for estimation of alcohol by dichromate method

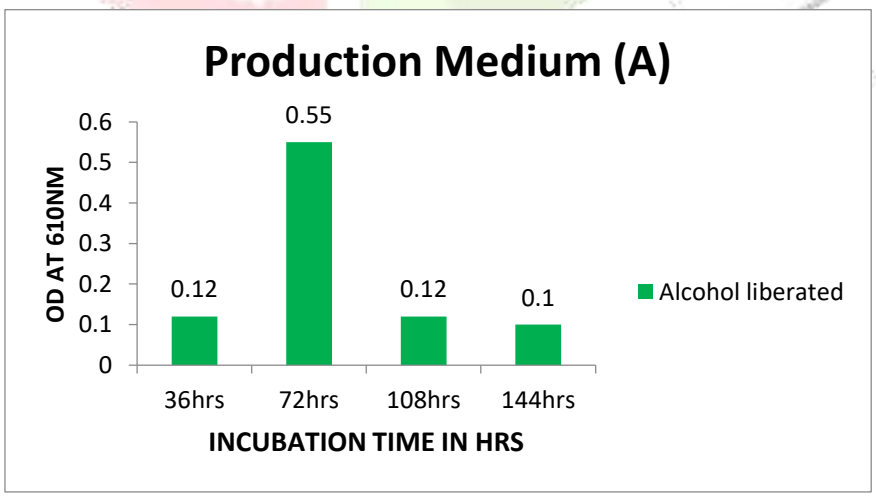
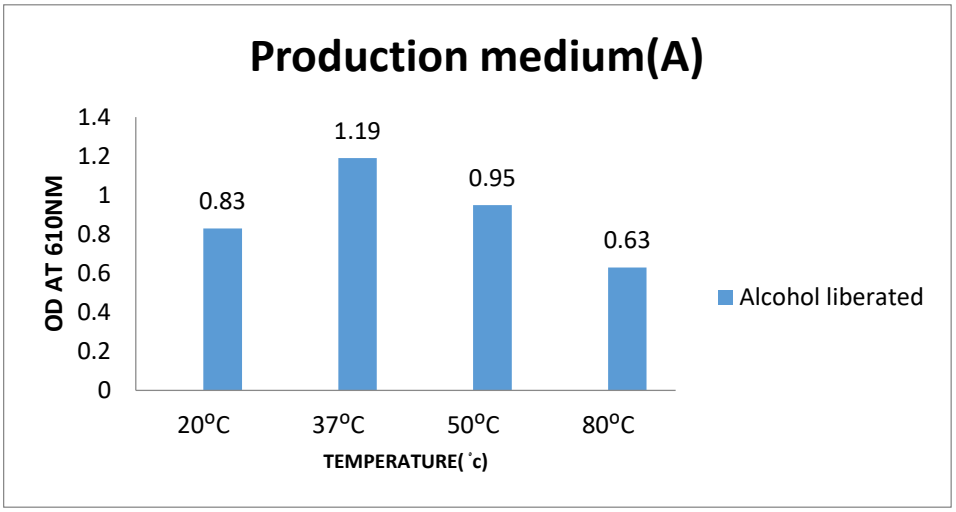


Fig 3.2: Effect of incubation period on estimation of alcohol in production medium (A)- Fruit waste



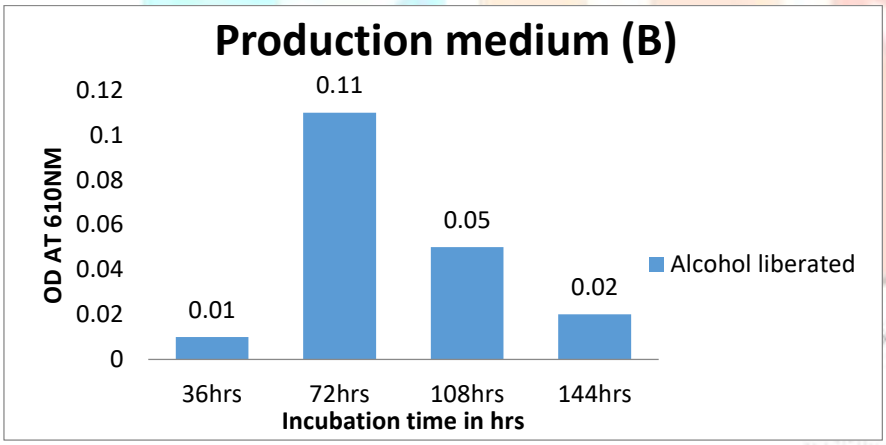


Fig 3.3: Effect of incubation period on estimation of alcohol in production medium (B)- vegetable waste

Fig 3.4 Effect of temperature on alcohol production in production medium (A)- fruit waste

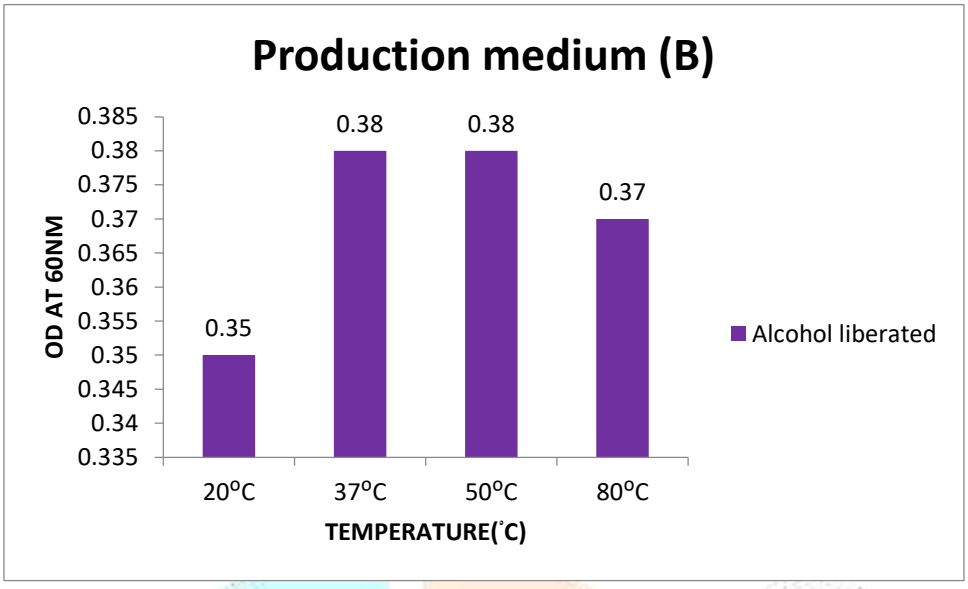


Fig 3.5 Effect of temperature on alcohol production in production medium (B)- vegetable waste

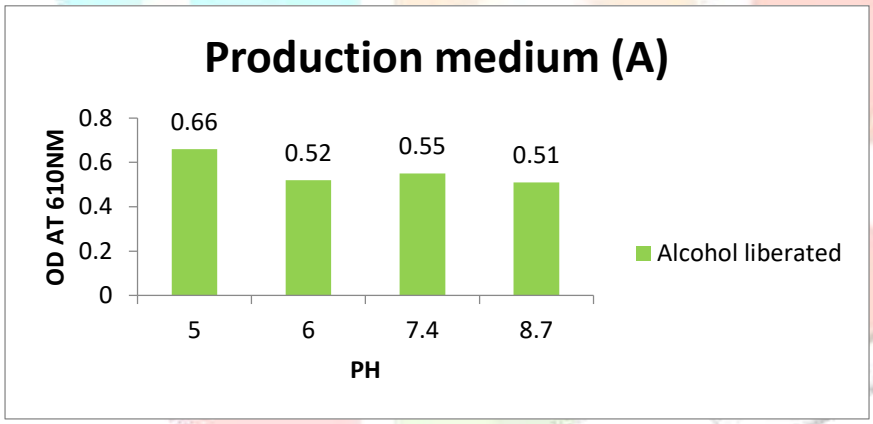


Fig 3.6 Effect of pH on alcohol production in production medium (A)- fruit waste

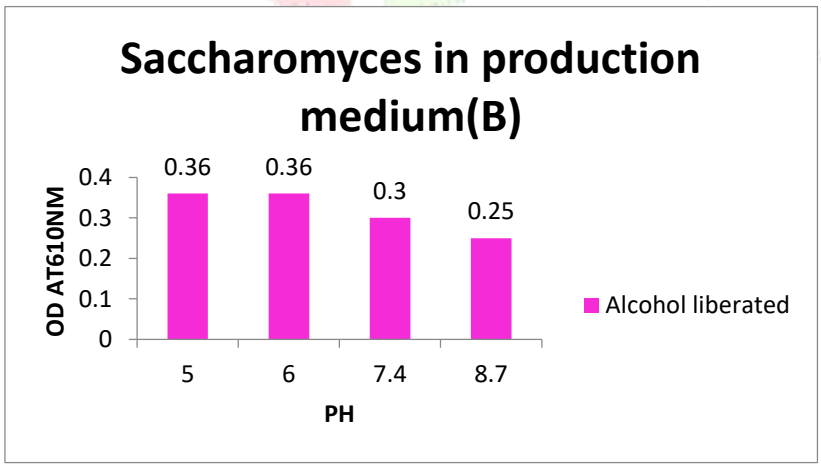


Fig 3.7 Effect of pH on alcohol production in production medium (B)- vegetable waste

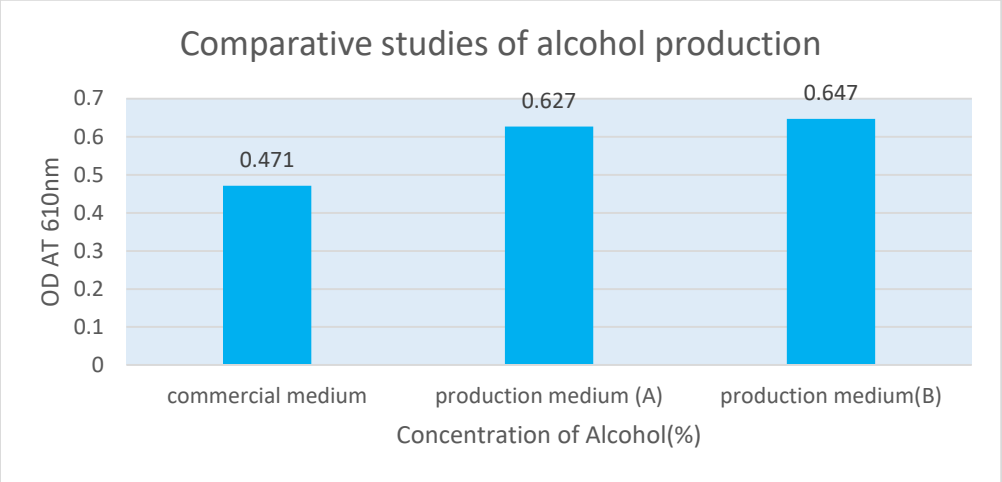


Fig 3.8: Comparative Studies Of Ethanol Production Using Fruit Waste And Vegetable Waste With commercial Medium

4.0 Discussion

Saccharomyces were used as selective organisms for ethanol production using fruit and vegetable waste which are considered to be a production medium A & B.

From our observation, it was found that the optimum production of alcohol was found to be at an incubation period of 72 hrs and the alcohol liberated was 0.55 % with fruit waste as substrate and with vegetable waste, it was 0.11%. Temperature had shown its tremendous effect on alcohol production by saccharomyces. Maximum enhancement was seen at the temperature of 37°c with the alcohol liberation of 1.19% with production medium (A) and minimum activity at 80°c with 0.63%and with production medium (B) the Conc. of alcohol was almost same at 37°c and 50°c. This may be due to slow breakdown of complex biomass molecules that are present in the vegetable waste. Minimum levels of alcohol production was o bserved at 20°c of 0.35% alcohol. pH plays a vital role in catalysis of enzymatic reaction next to temperature.yeast cells , using fruit waste had shown maximum levels of ethanol production at pH 5 with 0.66% and minimum at 8.7 with 0.51% surprisingly with the vegetable waste the organism didn't show much effect with the variations in the pH of 5 and 6 with the alcohol concentration of 0.36% and minimum activity at pH 8.7 of 0.25% Comparative studies had found that at temperature of 37°c and pH 5 saccharomyces cerevisiae produces high amounts of alcohol using production medium(B) with 0.647%, followed by Production medium (A) with 0.627% than with commercial medium of 0.471%. On comparision with commercial medium, fruit waste and vegetable waste are used as an excellent substrate for ethanol production on small-scale level. These kitchen wastes are also biodegradable after ethanol

5.0 Conclusion

Among the two tested sample,vegetable waste showed a slightly high levels of ethanol production of 0.647% at temperature 37°c and pH 5 by yeast cells than fruit waste. However, on comparison with commercial medium fruit waste is also an excellent substrate for ethanol production with 0.627%, which is equivalent to the vegetable waste.

From our present investigations we conclude that the biodegradable kitchen waste which includes both fruit waste and vegetable waste individually act as an excellent substrates for ethanol production and they act as an inexpensive raw materials thereby can reduce the fermentation economics. We anticipate that the byproduct that were formed will also be used as a biofertilizers.

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