Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2013, 4(5):119-123

ISSN: 0976-8610
CODEN (USA): AASRFC

Optimization and improvement of ethanol production by the incorporation of organic wastes

R. Thenmozhi and J. Victoria*

PG and Research Department of Microbiology and A Division of Biotechnology, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai, Mannargudi, Tamilnadu, India

---

ABSTRACT

Saccharomyces cerevisiae is the cheapest strain available for the conversion of biomass substrate. In the present study, it is used for bio-ethanol production from sugar molasses. Diluted cane molasses having total sugar and residual sugar content 7.0 and 7.14 % respectively was subjected to ethanol production by Saccharomyces cerevisiae. Incorporation of dried cauliflower waste and cabbage waste in molasses increased ethanol production at the level of 52.6% compared to molasses by nearly 40.8% alone. Addition of 20% yeast extract improved ethanol production upto 82% as compared to molasses alone. Cell biomass, ethanol production, final ethanol concentration and fermentation at 20% inoculum on 5 days respectively were found to be best at 10% Cabbage and cauliflower waste. The optimal value of the temperature, pH, yeast inoculum and fermentation period are found to be 35^{0}C, 5, 20% and 5 days respectively.

Keywords: Molasses, Cauliflower waste, Cabbage waste, Yeast extract, Ethanol production, Total residual sugars**.**

INTRODUCTION

Ethanol is a fuel, a solvent, an anti-freeze, and an organic feed stock in the chemical industry. It can be produced chemically from petroleum or microbiology from any fermentable carbohydrate by yeast. Economic factors weight heavily in choosing the method of ethanol production. When petroleum and natural gas prices are low, ethanol can be economically produced from petrochemical feed stocks; however, when petrochemicals are selling at a premium price, microbial production of ethanol from corn, molasses, or other plant material becomes more economical. When starch such as corn, and other complex carbohydrate are used as the raw material it is first necessary to hydrolyze them to simple fermentable sugars. The hydrolysis can be accomplished with enzyme from barley malt or molds or by heat treatment of acidified material. Corn, molasses, sugar beets, potatoes and grapes are some of the common raw materials employed throughout the world. Ethanol combustion produces lowered air population compared to gasoline combustion. At present, about 100 million gallons of ethanol per year are used as a fuel, but 12 million gallons per year would be required to completely replace gasoline use in the United States. Ethanol production from cellulosic materials by direct bioconversion is highly encouraging and its commercial production is established in countries like Brazil, Canada and USA. The economics of ethanol production by fermentation is significantly influenced by the cost of raw material, which accounts for more than half of the production cost. Ethanol contains 35% oxygen, which results in a complete combustion of fuel and thus lowers the emission of harmful gases. Converting a renewable non-fossil carbon, such as organic wastes and biomass consisting of all growing organic matter (plants, grasses, fruit waste and algae) to fuel would assure a continual energy supply[1].

Ethanol is an important chemical product with emerging potential as a biofuel to replace fossil fuels. An eco-friendly bio-ethanol is one of the alternate fuels that can be used in unmodified petrol engines with current fuelling infrastructure and it is easily applicable in present day combustion engine, as mixing with gasoline [2]. Bio-ethanol production from potatoes is based on the utilization of rotten potatoes are obtained from 5-20% of crops as by-products in potato cultivation[3]. The demand for ethanol has been on the increase due to its various uses such as,

chemical feedstock and more importantly as an alternative source of liquid fuel for automobiles. One of the ways of producing ethanol is through fermentation of crops which are rich in sugar or starch such as sugarcane, sugar beet, sweet sorghum, corn and cassava [5]. Cauliflower *(Brassica oleracae L*) is an important vegetable grown all over the world. The total cauliflower production in India in 2005 was 4.5 million tones which is about 28% of the total world production. Cauliflower has the highest wastes, ratio of edible portion to non edible portion and thus enormous amount of organic solid waste is generated. Submerged fermentation define does not have the problem of contamination, pH adjustment etc., which is a great problem in solid state fermentation [6].

MATERIALS AND METHODS

Collection of sample

Cauliflower and cabbage waste samples were collected from the local market in Orathanadu, Thanjavur (Dt), Tamil Nadu, and edible portion was removed and non-edible portion (CW) was oven dried, powered and retained for future trials. Cauliflower and cabbage were cleaned with sterile water, cut into small fractions of nearly 0.5cm manually and was completely dried at 50^0C in a hot air oven for 24 hours, ground using cyclotec sample mill. The powdered material was added into the medium in different concentration after moisture equilibration.

Collection of substrate

Molasses is the substrate for ethanol production. Molasses was collected from sugar cane molasses industry in Mannargudi by aseptic method.

Source or organisms

Saccharomyces cerevisiae MTCC 178 was procured from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India. The strain was maintained on YEPD medium containing (gl $^{-1)}$: yeast extract 0.75g; peptone 2.5g; dextrose 5g; agar 7.5g and stored in the refrigerator at 4-5^0C and was subcultured regularly at an interval of 15 days.

Fermentation medium [7]

Preparation of inoculum, *Saccharomyces cerevisiae* was transferred from agar slants into 250ml of Erlenmeyer flasks each containing 100ml of sterile YEPD broth and incubated at 30^0C for 24 hours or more till a required cell count was attained. The media used for fermentation are

a) 100% of molasses as control.
b) 80%of molasses + 20% 0f yeast extract.
c) 70% of molasses + 20% yeast extract + 10% cauliflower waste.
d) 70% of molasses + 20% yeast extract + 10% cabbage waste.
e) 60% of molasses + 20% yeast extract + 10% cauliflower waste + 10% cabbage waste.

100ml of the fermentation medium in 250ml Erlenmeyer flasks was used for experimentation purpose and the flasks were sterilized at 121^0C at 15 psi for 20 minutes. The initial pH of 5.0 was set for all the experiments.

The flasks were inoculated with 5% inoculum of yeast culture containing 1×10^8 cells\ml and were incubated at 28± 1^0C for 48 hours.

Determination of the Residual sugar

The residual sugar was determined using dinitrosalicylic acid (DNS) method described by [9].

DNS method

The sugar present in the sample reduce 3, 5 dinitrosalicylic acid to 3-amino-5- nitrosalicylate in the process sugar getting itself oxidized. The reduced salicylic acid gives an intense red colour whose intensity was observed[7]. Maximum is read at 540nm against a suitable blank.

Screening for ethanol

About 5 to 10ml of fermented sample was taken and pinch a potassium dichromate and a few drop of H_2SO_4 were added. The colour of the sample turns from pink to green which indicates the presence of bio-ethanol.

Estimation of ethanol [10]

Ethanol was determined after standard distillation using the method[8]. 5ml of the cauliflower and 5ml of the cabbage medium and 10ml double distilled water was taken in a flask to the drops of 1% phenolphthalein indicator was

added. Contents were mixed thoroughly, and then it was titrated against 0.1N NaOH until the persistence of pink colour.

The ethanol yield present in the sample was estimated by using the formula:

$$\% \text{ of ethanol} = \frac{(\text{Normality} \times \text{Volume of NaoH} \times \text{Molecular weight of NaoH})}{\text{Weight of sample (ml)} \times 10}$$

Optimization of pH [10].
100ml of molasses was prepared and dispensed into different flasks. The pH of the sterilized broth was set as 3, 4, 5, 6 and 7 in a flask. A loopful of *Saccharomyces cerevisiae* was inoculated into all the flasks. The flasks were incubated at 28^0C for 5days. After incubation the percentage of ethanol production was determined.

Optimization of Temperature [10]
100ml of molasses, cauliflower and cabbage was prepared and dispensed into different flasks. The temperature of the sterilized broth was set as 20^0C, 25^0C, 30^0C, 35^0C and 40^0C in a flask. A loopful of *Saccharomyces cerevisiae* was inoculated into the flasks and incubated for 5 days. After incubation, the percentage of ethanol production was determined.

Optimization of yeast concentration for ethanol production [9,10]
The optimum quantity of sugar molasses was solution was taken in fermentation flask and the pH and temperature were maintained at 5 and 35^0C. Various quantities of yeast like 1, 2, 4 and 8ml were added and kept for a period of 5 days and the fermented solutions were analyzed.

Cell Biomass
The dry cell mass of the sample was estimated by centrifuging the known volume of sample in a predried and preweighed centrifuge tube for 20 minutes. After resuspension in 2ml of distilled water and further centrifugation , the cell mass was dried. The dried cell mass was calculated by reweighing the tube [5,7].

RESULTS

The present investigation was carried out to study the yield of ethanol on cane molasses medium containing cauliflower and cabbage wastes. Molasses medium was analyzed for total reducing sugar (TRS). The percentage of TRS present in the molasses was adjusted to 8.5%. Ethanol production in the entire medium designed was screened by the production of green colour from pink using potassium dichromate method. No colour change was observed in the control. Ethanol was estimated by the methods[6]. In the molasses medium 35.8 g/l of ethanol was produced and the total residual sugar was 1.37 g/l. The decrease in the total residual sugar represents the utilization of sugars to get high ethanol yield. The cell biomass concentration was 1.98 g/l. In the medium supplementation with cauliflower waste, ethanol yield was 38.7 g/l. This reveals the improvement in the yield of ethanol. The cell biomass concentration was 2.37 g/l and the total residual sugar was 1.50 (Table-1). In the medium supplemented with cabbage waste the yield of ethanol was 40.8 g/l and the cell biomass and total residual sugar was 2.65 g/l and 1.99 g/l respectively, (Table-1). The medium supplemented with both cauliflower and cabbage wastes showed a considerable increase in the yield of ethanol as 52.6 g/l, the cell biomass concentration of 2.5 g/l and the total residual sugar of 2.45 g/l (Table-1). From these results the supplementation of cauliflower and cabbage wastes resulted high yield due to the increased concentration of minerals, essential vitamins and amino acids which in turn stimulated the yeast cell growth and subsequent ethanol producing capability.

Fermentation medium prepared were placed in different temperature (20^0C, 25^0C, 30^0C, 35^0C, and 40^0C) to analyze the optimum temperature for ethanol production with the pH of 5 and 2% yeast cell concentration. At the temperature of 35^0C, maximum yield of ethanol 84.3g/l was observed when compared to other temperatures (Table-1). The pH of the fermentation medium thus prepared was adjusted to 3, 4, 5, 6, and 7s with the temperature of 35^0C and 20% yeast. Maximum yield of ethanol 80.3 g/l was obtained at a pH of 5. In the acidic pH the productivity of the organism was high and maximum cell biomass was also obtained, (Table-2). Different concentration of inoculum was (10%, 20%, 30%, 40% and 50%) added to the entire fermentation medium with pH 5 and a temperature of 35^0C. Maximum with yield 82 g/l was obtained at 20% concentration of the inoculums when compared to others. As the concentration increases the yield was decreased hence the optimum concentration of yeast cell was assigned as 20% for highest ethanol production (Table-3).

Thus the maximum yield of ethanol was obtained at the pH and temperature and inoculum concentration of 5, 35^0C and 20% respectively for 5 days of incubation. From this study, we conclude, that for maximum ethanol production

leafy vegetables waste substrates can be utilized, so that the enormous dumping of the wastes and the rate of pollution can be overcome. Therefore it is also a cost effective method for the production of ethanol by which, the wastes can be reused. In the present study, highest yield of ethanol was achieved by incorporating the leafy vegetable wastes such as cauliflower and cabbage wastes into the fermentation medium.

Addition of yeast extract (0.2%, w/v) into the medium containing molasses and different levels of cauliflower stimulated ethanol production both in terms of reduction in time and increased efficiency for all the treatments [10]. Our results are also in line with the above said observations like incorporation of cauliflower and cabbage wastes in the medium stimulated the ethanol production.

Table-1: Production of Ethanol at the optimized conditions

S.No	Treatment	Cell biomass (g/l)	Ethanol (v/v)	Total residual sugar (g/l)
1	Molasses	-	-	1.24 ± 0.24
2	Molasses + yeast extract	1.87 ± 0.54	**35.8** ± 1.11	**1.36** ± 0.31
3	Molasses + yeast extract + cauliflower waste	**2.35** ± 0.3	**38.7** ± 0.92	**1.51** ± 0.24
4	Molasses + yeast extract + cabbage waste	**2.63** ± 0.44	40.8 ± 1.02	**1.69** ± 0.32
5	Molasses + yeast extract + cauliflower waste + cabbage waste	**2.55** ± 0.43	**52.6** ± 1.26	**2.46** ± 0.66

Values are expressed as Mean ±Standard Deviation

Table-2: Effect of pH on ethanol production

S.No	Treatment	pH				
		3	4	5	6	7
1	Molasses	**65.0** ± 2.0	**61.8**±1.22	**69.0**±2.0	**60.0**±2.0	**60.0**±1.87
2	Molasses + yeast extract	**71.0**±1.5	**70.3**±1.58	**73.0**±1.73	**67.3**±1.58	**67.0**±2.23
3	Molasses + yeast extract + cauliflower waste	**68.0**±1.4	**66.3**±1.22	**75.0**±1.58	**65.3**±1.56	**63.3**±1.56
4	Molasses + yeast extract + cabbage waste	**73.0**±2.0	**71.6**±1.4	**79.0**±1.73	**70.6**±2.0	**67.3**±1.58
5	Molasses + yeast extract + cauliflower waste + cabbage waste	**76.0**±1.73	**72.6**±1.58	**80.3**±1.58	**76**±1.87	**71.3**±1.87

Values are expressed as Mean ±Standard Deviation

Table-3: Effect of temperature on ethanol production

S.No	Treatment	Temperature				
		20	25	30	35	40
1	Molasses	**69.3** ± 2.0	**67.6**±2.12	**65.0**±2.23	**75.6**±2.34	**63.0**±2.0
2	Molasses + yeast extract	**72.0**±2.1	**70.3**±1.87	**69.0**±1.58	**78.3**±2.12	**68.2**±4.0
3	Molasses + yeast extract + cauliflower waste	**73.6**±1.58	**71.6**±1.73	**70.0**±2.23	**76.2**±0.34	**71.6**±2.0
4	Molasses + yeast extract + cabbage waste	**71.0**±3.0	**73.3**±2.0	**71.3**±1.73	**79.3**±1.58	**69.6**±1.87
5	Molasses + yeast extract + cauliflower waste + cabbage waste	**79.6**±1.22	**77.0**±2.12	**76.3**±1.87	**84.3**±2.12	**80.6**±2.12

Values are expressed as Mean ±Standard Deviation

DISCUSSION

Our results both in terms of cell biomass, ethanol production and fermentation time are in consonance with the results of Reddy and Reddy who have reported a 50% increase in ethanol production and reduction in fermentation time while producing ethanol from dried mango peel by the use of yeast extract in fermentation medium [9]. Likewise in this study supplementation of cauliflower and cabbage wastes increased the ethanol concentration and fermentation rate. In the cauliflower waste supplemented medium, 38.7 g/l yield of ethanol was obtained. In the cabbage waste, yield of ethanol was 40.8 g/l. Also in the medium supplemented with both cauliflower and cabbage waste showed a significant increase in the yield of ethanol (52.6 g/l).

In this study both *Z.mobilis* and *S. cerevisiae*, exhibited potential for ethanol production. However, they could be manipulated genetically for higher ethanol tolerance/production[3]

Disposal of this nutritionally rich cauliflower in municipal bins results in rotting which creates foul smell thereby adding to the environmental problems and jeopardizes public health. Due to its nutritional value, these wastes can be utilized as important substrates for production of industrially important products such as bio-ethanol or enzymes. The rapidly depleting fossil fuels and constant rising crude oil prices all over the world has increased interest in alternative source of energy [7].

Ethanol fuel is widely used in Brazil and in the United States. Both countries were responsible for 87.1% of the world's ethanol fuel production in 2011[8].

Particularly, cauliflower has the highest waste ratio of edible portion to non-edible portion and thus enormous amount of organic solid waste is generated and also one million tons of cabbage wastes are discarded per year. So to reutilize these wastes in a proper way to reduce pollution and economic loss, they can be incorporated in the medium used for ethanol production in large scale [7,9].

REFERENCES

[1] Wyman,CE Spindler,DD and Gromann,K, *Biomass.Bioenergy*. **1992,** 3(5), 301-307.
[2] Adarsha,A Asha,DL and Balaji,*RR,.African journal of Microbiology Research.***2010**, 4(12) , 1340-1342.
[3] Akponah E., Akpomie O. O. and Ubogu M, *European Journal of Experimental Biology*, **2013**, 3(4),247-253.
[4] Hansen Alan,C Qin Zhang, Peter and Lyne,WL, *Biotechnol.Bioeng.* **2005**, 40,752-759**.**
[5] AOAC and D.C Washington, *Official Methods of Analysis*, 13th edition, Association of Official Analytical Chemist, **1974.**
[6] Kaliz,HM. *Adv.Biochem.Engg*, **1988.** 3(1)**,** 51-62.
[7] Miller,GC. *Analytical Chemistry***,1959,** 35-62
[8] Nitesh Kumar, Jai Prakash Singh, Ravi Ranjan, Subathradevi C. and Mohana Srinivasan V, *Advances in Applied Science Research*, **2013**, 4(4),299-302.
[9]Okolo,BN Ezegu,LI and Ebisike,CO, *World Journal of Microbiology and Biotechnology***.1995**, 12(6),637-638**.**
[10] Sadasivam,S and Manickam,A, *Biochemical Methods*, 2nd edn, New Age International Publishers, New Delhi, **1996**.