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OPTIMIZATION OF YEAST FOR ETHANOL PRODUCTION

Taghizadeh Ghassem[1], Delbari Azam Sadat[2] and Kulkarni D. K.[3]*
[1]Department of Microbiology, Bharati Vidyapeeth University, Maharastra, India
[2]Department of Environmental Science, University of Pune, Maharastra, India
[3]BAIF Development Research Foundation, Pune, Maharastra, India

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***Corresponding author**
Dr. D. K. Kulkarni, BAIF Development Research Foundation, Pune 411 058 Maharastra, India
Email: dilipkkulkarni@gmail.com

ABSTRACT
The production of pure ethanol apparently begins in the 12-14th century. Improvements in the distillation process with the condensation of vapors of lower boiling liquids. Ethanol is produced commercially by chemical synthesis or biosynthesis. High ethanol producing yeast exhibits rapid metabolic activity and a high fermentation rate with high product output in less time.
Yeasts were isolated from Corn, Curd, Grapes, Water 1, Water 2, and Paneer. Isolation was done on MGYP (Malt Extract Glucose Yeast extract Peptone) media. Contamination was less in selected media. Grape sample yeast was observed as high in producing ethanol after optimization in jaggery broth. Curd yeast gives 4.6% alcohol by volume alcohol (a.b.v) after fermentation .Paneer yeast gives 2.88% alcohol by volume alcohol (a.b.v) after fermentation. Corn yeast gives 5.25% (a.b.v) alcohol after fermentation Water-1 yeast gives 5.51% (a.b.v) alcohol after fermentation.Water-2 yeast gives 4.98% (a.b.v) alcohol after fermentation.
Keywords: Yeast, Ethanol, Corn, Grape, Paneer

INTRODUCTION

During the 18th century alcohol was not only used for the production but also as a constituent of medical drugs and manufacture of paint pigments and other industrial chemicals. It was initiated in the 19th century and the trade became an industry with enormous production[1]. Ethanol is an important industrial chemical with emerging potential as a bio-fuel to replace fossil fuels.[2]

Ethanol is produced by chemical synthesis using hydration of ethylene (C_2H_4). Biosynthesis is the fermentation process and yeast monosaccharides as a carbon source and then converts to ethanol via glycosis under anaerobic conditions.[4]

Glucose Ethanol at the beginning of the 20th century, several kinds of raw materials were exploited for ethanol production, such as molasses or agricultural produce and the possibility of hydrolyzing lignocellulosic materials was investigated[12]. Carbohydrate-rich raw materials suitable for ethanol production are classified into three groups of agricultural products: sugars, starch and lignocellulose. The first raw material group is from sugar like sugar-beet, sugarcane and molasses. The second group is starch from crops includes cassava, cereals and potatoes. The last group is lignocelluloses which cover waste materials from the agriculture crops such as rice straw, corn cob and sugarcane waste[11]. There are several wild relatives of grapes mentioned in the literature[10]. However, the yeast is used only in grape (*Vitis vinifera* L.). In general, industrial yeast strains are able to grow and efficiently fermented ethanol at pH values of 3.5-6.0 and temperatures at 28-30 ^{0}C. There are several potential benefits of high ethanol producing yeast in the production of industrial alcohol[9].

High ethanol producing yeast exhibits rapid metabolic activity and a high fermentation rate with high product output. Present paper deals with high ethanol production by using biological material.[3]

MATERIALS AND METHODS

Isolation and screening of yeasts

Yeasts were isolated from Corn, Curd, Grapes, Water1, Water 2, and Paneer.

Each sample was processed as follows:

1. Sample was taken to cleaned Laminar Air Flow cabinet.
2. Under aseptic conditions, 1gr of homogenized sample was added to 20 ml of MGYP broth.
3. Sample was incubated at 26 °C for 2 days.

1. Corn; Corn sample was washed in sterile D/W and then crushed in sterilized pestal and mortal. Then approximately 2gr of crushed sample was inoculated in 10 ml MGYP broth aseptically.

2. Curd; 1ml Homogenized curd sample was inoculated in 9 ml of MGYP broth aseptically.

3. Grapes; Grape sample was washed using sterile D/W and crushed. Approximately 1g of sample was aseptically inoculated in 10 ml of sterile MGYP broth.

4. Water sample 1; Approximately 1ml of dirty water (collected from hotel in Laxmei road) sample was aseptically inoculated in 9 ml of sterile MGYP broth.

5. Water sample 2; Approximately 1ml of dirty water (collected from hotel in Shvaji station) sample was aseptically inoculated in 9 ml of sterile MGYP broth.

6. Paneer; Paneer sample was homogenized in sterile D/W and approximately 1ml of sample was aseptically inoculated in 9 ml MGYP broth

Staining of yeast culture to confirm presence of yeast cells

Loopfull of enrichment was removed from each sample tube and staining was done by Monochrome staining. Staining was performed as per standard procedure[9].

Isolation of yeast on MGYP media

1. A loop ful culture from the enrichment samples showing yeast cell was aseptically removed and streaked MGYP media by four quadrant method.
2. Plates were incubated for 2 days at 26 ^{0}C.

3. Single colonies from each plate were observed for colony characteristics.
4. Single colonies were picked and suspended in sterile saline tubes.
5. Monochrome staining was performed to check purity of the colony.
6. Same procedure was repeated till pure cultures were obtained from each sample.

Ethanol production in jaggery broth

Protocol for ethanol production in jaggery broth

- Preparation of jaggery broth
- Inoculum preparation
- Incubation at 30 ºC. and at 200 rpm for 48 hours
- Sterilization of media (jaggery broth) at 121 ^{0}C for 15 min at 15 PSI pressure
- Inoculation of 48 hour old cultures of yeast varieties in jaggery broth
- Incubation at 27-30 ^{0}C for 7-8 days
- Filtration of broth with muslin cloth or cotton
- Determination of alcohol content in each filtrate by hydrometer

Inoculums preparation

50 ml of jaggery broth was poured in 8 sterile bottles and autoclaved at 121 ^{0}C at 15 psi for 20 minutes. 1-2 colonies from pure yeast cultures were aseptically inoculated in autoclaved jaggery broth. These bottles then kept on shaker for 48 hours at 30 ^{0}C for 2 days.

Sterilization of jaggery broth in beer bottles

About 450 ml of jaggery broth was poured in 8 bottles. Then all the 8 bottles were autoclaved at 121 ^{0}C at 15 psi for 20 minutes.

Inoculation of 48 hour old inoculate in fermentation media

48 hr old inoculate was poured aseptically in beer bottles and bottles were kept at 27-30 ^{0}C for 7 days.

Filtration of fermented broth through sterile cotton

All the 8 fermented broths were filtered with the help of sterile cotton and funnel. Filtrate was again poured in the new bottles.

Determination of alcohol content in each filtrate by hydrometer

1. Take about 60 ml initial jaggery broth (before inoculation of yeast) in 100 ml measuring cylinder.
2. Put the hydrometer in the 60 ml initial broth containing cylinder.
3. Take the reading as a initial specific gravity of jaggery broth.
4. Take the fermented jaggery broth of any one sample and repeat the same procedure.
5. Now take this reading as final specific gravity of fermented jaggery broth.
6. Then repeat the procedure for all jaggery broths.
7. Now do the calculations according to the hydrometer calculation chart.

RESULTS AND DISCUSSION

Enrichment of sample

After strike sample on MGYP media and were picked single colony to check the morphology characteristic. All samples showed growth in the form of turbidity. Table 1 shows morphology characteristics of yeast isolates.

After incubation seven isolates were obtained, colony characters were checked and stain had been done. Table 2 showed results of colony characteristics of yeast isolates. Sizes of all colonies were different while shapes of colonies were spherical with white color. Margin of all colonies were entire also colonies elevation were concave except S-2 colony that was plane. Opacity of all colonies were showed opaque with dry consistency except colonies of paneer and grape that were moisture.

Table number 3 showed result of alcohol produced by yeast isolates. The yeast isolated from curd and corn were 4.6% alcohol by volume (a.b.v) 5.25% (a.b.v), respectably. The yeast isolated from paneer was very less. Grape yeast isolate was 6.03% (a.b.v) alcohol after fermentation. Out of all sample of yeast isolates, grapes gave highest alcohol production.

Ethanol fermentation is responsible for the rising of bread dough. Yeast organisms consume sugars in the dough and produce ethanol and carbon dioxide as waste products. The carbon dioxide forms bubbles in the dough, expanding it into something of foam. Nearly all the ethanol evaporates from the dough when the bread is baked.

All alcoholic beverages, including those produced by carbonic maceration are produced by ethanol fermentation by yeast. Wine and brandy are produced by fermentation of the natural sugars present in fruits, especially grapes.

Grape is used to make wine in many countries.[5] Grape juice can be easily stored, allowing winemakers to produce wine throughout the year. Concentrate is more resistant to spoilage and is less expensive to handle and transport than non-concentrated grape juice[6]. The ferment ability of grape is related to cultivar, environment, fertility of soil, conditions of maturity at harvest and treatment of grapes in the winery.[7, 8]

In present study, curd, corn, paneer and grape were yeast isolated but highest yeast isolate gave 6.03% (a.b.v) in grape alcohol after fermentation. This isolate observed that the yeasts are normally large in size and they may be round or rod shaped. Grape yeast was round in shaped whose colonies were dry and opaque and their size is about 0.7mm.

The methods used in research suggested that high alcohol produced directly from grape, which will be useful for ethanol production in many locations. Results indicate that grape sample yeast was observed as highest ethanol producing yeast after optimization in jaggery broth. Better production of ethanol, temperature, nutrient and pH on grape yeast must be optimized .

Table1: Morphology characteristics of Yeast isolates

SN	Name of the sample	Morphology observed
1	Grapes	Big and round shaped violet coloured cells
2	Corn	Long and rod shaped violet coloured cells
3	Curd	Small and round shaped violet coloured cells
4	Paneer	Small and oval shaped violet coloured cells
5	Water-1	Big and round shaped violet coloured cells
6	Water-2	Big and oval shaped violet coloured cells

Table 2: Colony characteristics of Yeast isolates

Sr. No.	Colony Characteristics	Water 1	Paneer	S-2	S-1	Water2	Grape	Curd
1	Size	0.5mm	0.6mm	0.7mm	0.5mm	0.6mm	0.7mm	0.5mm
2	Shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
3	Color	White	White	White	White	White	White	White
4	Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire
5	Elevation	Concave	Concave	Plane	Concave	Concave	Concave	Concave
6	Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
7	Consistency	Dry	Moisture	Dry	Dry	Dry	Moisture	Dry

Table3: Percent of alcohol produced by yeast isolates

NO.	Name of sample	Specific Gravity	A.B.W.	A.B.V.
1	Curd	1.025	3.68%	4.6%
2	Paneer	1.038	2.31%	2.88%
3	Corn	1.020	4.2%	5.25%
4	Water-1	1.018	4.41%	5.51%
5	Water-2	1.022	3.99%	4.98%
6	Std-1	1.015	4.72%	5.9%
7	Std-2	1.010	5.25%	6.56%
8	Grapes	1.014	4.83%	6.03%

Note; A.B.W → alcohol by weight and A.B.V → alcohol by volume

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