ejpmr, 2020,7(11), 397-399

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PRODUCTION OF ETHANOL FROM *SACCHAROMYCES SP* BY USING CORN COB THROUGH SUBMERGED FERMENTATION

Roopa B.*, Arbin Sultana, Arpitha H. B., Afreen Banu M., Anithalakshmi B., Karishma K. B. and Ruheena Khanam

Department of PG Studies and Research in Botany, Tumkur University, Tumkur-572106.

***Corresponding Author: Roopa B.**

Department of PG Studies and Research in Botany, Tumkur University, Tumkur-572106.

Article Received on 01/09/2020 **Article Revised on 21/09/2020** **Article Accepted on 11/10/2020**

ABSTRACT

The utilization of ethanol as an alternative fuel has escalated recently because of some conceivable reasons. Bioethanol can be produced by fermenting sugars from biomasses and used as fuel for vehicular engine internal combustion. Currently, most of the conventional ethanol fermentation feedstocks are relying on agricultural crops, such as starch-based crops and sugar-based crops. *Saccharomyces sp* were isolated from different fruits, including (i) White Grapes, (ii) Black grapes, (iii) Sapota. The isolated *Saccharomyces sp* were screened for production by qualitative method. Ethanol were produced by using corn cob as substrate through fermentation process. Optimization were studied by employing pH, temperature and inoculum size. The pH 5.0 showed 1.03% of ethanol production. The temperature (30^0C) and inoculum size (1.0 ml) showed 1.23% and 1.54% of ethanol were produced.

KEYWORDS: Bioethanol, Corn cob, *Saccharomyces sp* , optimization studies and Submerged fermentation.

INTRODUCTION

The global community has acknowledged biofuel (bioethanol) for providing energy security, thereby reducing the dependence on fossil fuels. Bioethanol is the dominating biofuel for transportation, with an annual world production increasing from 28.5 million m^3 in 2004 to 87.2 million m^3 in 2013. (REN21, 2014). Bioethanol (C_2H_5OH) is a liquid biofuel, produced from several different biomass feed stocks, using various conversion technologies. It is an attractive alternative fuel, as it is renewable, bio-based, and oxygenated (35% oxygen), hence providing a potential to reduce particulate and NOx emissions in compression-ignition engines (Balat, 2007 and Hsieh, et al., 2002).

Saccharification of agricultural by-products can be done either through acid hydrolysis or enzymatic hydrolysis. Taherzadeh and Karimi (2007) reported that enzymatic hydrolysis is more beneficial than acid hydrolysis. This is due to the absence of sugar degradation into Hydroxy Methyl Furfur aldehyde (HMF) or furfural, milder reactions (low temperature, neutral pH), potential for high results in a reaction, and low maintenance expense (no corrosive instruments are used). Zhao *et al*. (2008) had also stated that enzymatic hydrolysis only uses low energy input, has low pollution effects and no side products such as furfural or HMF are detected.

The present study highlights on ethanol (bioethanol) production by *saccharomyces sp* by using corn cob as a substrate. The *saccharomyces sp* were isolated from fruit samples and screened for ethanol production and confirmed *saccharomyces sp* were used for optimization studies for ethanol production.

MATERIALS AND METHODS

1. Isolation and Cultivation of Yeast

Yeast culture were isolated from different fruits, including (i) White Grapes, (ii) Black grapes, (iii) Sapota. About 0.5 gram of each fruits were used for yeasts source and further serially diluted in saline solution until 10^{-3} to $10^{-4}$of dilution. About 100 µl of each of the serially diluted sample was then spread on top of Yeast-extract, Peptone and Dextrose (YEPD). The plates were incubated at room temperature for 5-7 days.

2. Corn Cob as Substrate for Ethanol Production

Take 25 g of the Corn cob sample and make into small pieces and put into 500 ml beaker containing 250 ml double distilled water. The flak were kept for boiling for an hour and after boiling cool and filter the boiled sample and filtrate were used for further fermentation studies.

3. Fermentation Medium

250 mL Erlenmeyer flasks contained 100 mL of Corn cob extracted fermentation media were prepared and initial pH of the media were adjusted. Consequently prepared flasks were cotton plugged and sterilized by autoclave at 15 lbs, 121^0C for 15 min. The flasks were

aseptically inoculated with freshly prepared *Saccharomyces sp* were inoculated and incubated.

4. Qualitative Test for Ethanol

The 2 ml of acetone was added to a test tube containing 4 drops of the fractionated bioethanol. 2 drops of chromic acid were then added. The mixture was shaken vigorously.[1-3] The change in colour of the mixture forming a blue-green precipitates within few second of adding drops of chromic acid confirms the presence of ethanol (Tambuwal Amamatu Dahiru, 2018).

5. Production of Bioethanol with Optimization

The production medium were prepared from Corn cob extract. The corn cob extracts were used for the optimization studies by using *Saccharomyces sp*.

a. Effect of pH on Biosynthesis of Bioethanol

250 mL Erlenmeyer flasks contained 100 mL of corn cob extract were prepared and initial pH of the production media were adjusted. The adjusted initial pH of fermentation media were ranging from 3-7 with increments of 1.0. Consequently prepared flasks were cotton plugged and sterilized by autoclave at 15 lbs, 121^0C for 15 min. The flasks were aseptically inoculated with freshly prepared spore suspension and incubated.

b. Effect of temperature on Biosynthesis of Bioethanol

100mL of the corn cob extract were used for production of bioethanol and media were collected separately in 250 ml Erlenmeyer flasks and prepared for submerged fermentation. Thus prepared flasks were incubated at different temperatures like 25^0C, 30^0C, 35^0C and 40^0C.

c. Effect of Inoculum size on Biosynthesis of Bioethanol

The inoculum was prepared by 168h freshly prepared culture of *Saccharomyces sp* at different levels i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 mL and then inoculated and fermentation studies were carried out.

6. Estimation of Bioethanol Production

Quantitative estimation of ethanol

Most of the chemical oxidation methods are based on the complete oxidation of ethanol by dichromate in the presence of sulphuric acid with the formation of acetic acid.

The theoretical reaction stoichiometry is shown below:

$2Cr_2O_7$-- + $3C_2H_5OH$ + 16H+ -----> $4Cr^{+++}$ + $3CH_3COOH$ + $11H_2O$

10-50 microlitres of absolute alcohol was taken in different test tubes and the volume was made up to 500 microliters by adding distilled water in each test tube.30 microlitres of test sample was taken and the volume was made up to 500 microliters by adding distilled water in test tube.1 ml of potassium dichromate reagent was added in each test tube. Then 2 ml of sodium hydroxide solution was added in each test tube. The test tubes were incubated at 500C for 30 minutes. The absorbance was measured at 600 nm by using a spectrophotometer.(Nair Sreecha Chandran et al., 2018).

RESULTS AND DISCUSSION

The *Saccharomyces sp* were isolated from different fruit samples from different Market places in Bangalore. The fruits were used are, white grapes, black grapes, and sapota. The isolated *Saccharomyces sp* were screened for ethanol production by qualitative method and confirmed the production of ethanol (Tambuwal Amamatu Dahiru et al., 2018).

Optimization studies were carried out by optimizing pH, temperature and inoculum size. The results obtained in the study on the effect of initial pH in submerged fermentation for ethanol production by *Saccharomyces sp* is represented in Table-1. It reveals that the ethanol production were increased by the increasing of pH of the medium from pH 3.0 to pH 5.0 and then further increase in initial pH caused the declining of ethanol production. These increasing peaks were observed up to 48 hours of fermentation period and thereafter the decreased yield as fermentation period increased. The maximum ethanol production showed 1.03% was obtained at pH 5.0 for 48 hours of fermentation period.

Effect of temperature on biosynthesis of ethanol were also studied and represented in table-2. The ethanol production reveals that the increase of temperature of the medium from 25^0C, up to temperature 30^0C with optimized constant pH of 5.0 shows increased yield of ethanol bu using *Saccharomyces sp*. These increasing peaks were observed up to 48 hours of fermentation period and thereafter the decreased yield as temperature levels and fermentation period increased. The maximum ethanol production showed 1.23% was obtained at temperature 30^0C for 72 hours of fermentation period.

The inoculum size on ethanol synthesis results were obtained in submerged fermentation of by *Saccharomyces sp* is represented in Table-3. Out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 mL), 1.0 mL inoculum was found to be the most suitable for high production of ethanol by *Saccharomyces sp* in submerged fermentation at 48 h of fermentation and it showed 1.54%.

Seer et al., (2016) reported that the ethanol production were influenced with pH 5.0 showed better yield of ethanol. In general, H+ concentrations in fermentation broth can change the total charge of plasma membrane affecting the permeability of some essential nutrients into the cells. The optimum pH range for *S. cerevisiae* used in fermentation for ethanol production is 4.0–5.0. However, very recently, it was reported that this well-known yeast could produce ethanol from date juices even at pH 3.8, though the critical pH for this organism is 2.3. On the other hand, the highest ethanol yield was obtained using

Z. mobilis adjusting the pH range of the broth as 5.0–6.0. (Hossain Zabed et al., 2014).

Seer et al., (2016) were also reported that the temperature 35^0C showed better yield of ethanol. In a study with sweet sorghum juice using immobilized yeast cells, it was reported that at 28∘ C ethanol yield was 75.79% followed by growing up to the maximum yield (89.89%) at 37^0C. In another study with the strain *S. cerevisiae* BY4742 in batch fermentation, Lin et al., reported that the highest specific cell growth rate and specific productivity of ethanol were found at 30–45 ^{0}C with a significant decrease in cell growth as well as in ethanol yield at 50∘ C. In case of Z. mobilis, the best ethanol concentration (55.57 g/L) was found at 30 ^{0}C, while the lowest (4.6 g/L) was found at 40 ^{0}C (Hossain Zabed et al., 2014).

Inoculum concentration does not have significant influence on final ethanol concentration but significantly affects sugar consumption rate and ethanol productivity. However, it was reported that ethanol production was increased with the increase in the initial cell numbers from 1 × 104 to 1 × 107 cells/mL and no significant difference in ethanol production was found between 107 and 108 cells/mL (Hossain Zabed et al., 2014).

Table-1: Effect of pH on Ethanol production.

pH				
	Day 1	**Day 2**	**Day 3**	**Day 4**
3	0.42%	0.65%	0.39%	0.24%
4	0.54%	0.85%	0.68%	0.31%
5	0.68%	1.03%	0.98%	0.59%
6	0.58%	0.73%	0.48%	0.36%
7	0.52%	0.63%	0.43%	0.35%

Table-2: Effect of temperature on Ethanol production.

Temperature				
	Day 1	**Day 2**	**Day 3**	**Day 4**
25	0.34%	0.46%	0.38%	0.24%
30	0.68%	1.23%	0.72%	0.56%
35	0.36%	0.68%	0.53%	0.42%
40	0.32%	0.55%	0.45%	0.28%

Table-3: Effect of inoculum size on Ethanol production.

Inoculum Size				
	Day 1	**Day 2**	**Day 3**	**Day 4**
0.25	0.25%	0.67%	0.44%	0.31%
0.50	0.36%	0.86%	0.62%	0.47%
0.75	0.44%	0.76%	0.60%	0.34%
1.00	0.69%	1.54%	1.12%	0.68%
1.25	0.53%	1.22%	0.81%	0.50%

REFERENCE

1. REN21, Renewables Global Status Report, REN21 Secretariat, 2014.
2. Balat, M. Global bio-fuel processing and production trends, Energy, Exploration & Exploitation, 2007; 25(3): 195-218.
3. Hsieh, W.-D. Chen, R.H, Wu, T.L. Lin T.H Engine performance and pollutant emission of an SI engine using ethanol gasoline blended fuels, Atmospheric Environment, 2002; 36(3): 403-410.
4. Tambuwal Amamatu Dahiru, Muhammad Inusa Babanladi, Alhaji Sani, Muhammad Sirajo and Ogbiko Cyril. Production and characterization of bioethanol from *Solanum lycopersicum* stalk hydrolysates by the simultaneous saccharification and fermentation using *Zymomonas mobilis* and *Saccharomyces cerevisae*, 2018.
5. Nair Sreecha Chandran, Sonia John, Parvathy G, Vismaya N Kumar, Ruchitha R. Ethanol Production From News Paper Waste, 2018; 6: 458-463**.**
6. Seer, Q H Nandong, J and Shanon, T Experimental study of bioethanol production using mixed cassava and durian seed. 29th Symposium of Malaysian Chemical Engineers.IOP publishing, 2016; 1-7.
7. Hossain Zabed, Golam Faruq, Jaya Narayan Sahu, Mohd Sofian Azirun, Rosli Hashim, and Amru Nasrulhaq Boyce. Bioethanol Production from Fermentable Sugar Juice Scientific World Journal, 2014; 1-11.