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Cost-effective production of bioethanol from low-quality apples by using *Saccharomyces cerevisiae*

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ABSTRACT

Bioethanol production at the industrial level is engaged by various bacteria, yeast, and fungi. But *Saccharomyces cerevisiae* is the most commonly used yeast. Different substrates have been used for ethanol production such as sweat sorghum cane extract, lignocellulose, starch-based substrate and molasses, and other wastes. In this study low-quality apples were used for the production of ethanol that was further used for various purposes. From this work, it is clear that the maximum ethanol production is obtained from the apples. *S. cerevisiae* strain was used for the maximum conversion of sugar contents of apple extracts into ethanol. Different parameters like incubation time, temperature, pH and Inoculum size were optimized to get the maximum ethanol production that can be increased after further studies and proved to be a beneficial candidate for different applications. Maximum ethanol (13.9 %) was produced after inoculation was performed by *S. cerevisiae* (inoculum size 4%). The optimized conditions of apple extracts inoculated with *S. cerevisiae* at 30°C after 72 hours of incubation having pH of about 4.0.

Keywords: Bioethanol, fermentation, apples, *Saccharomyces cerevisiae*

INTRODUCTION

For the production of biofuel, utilisation of renewable lignocellulosic biomass sources has been planned as an appropriate option to deal with the depletion of fossil fuels and the improvement of climate. First-generation biofuels are those made from starch-based feedstocks, grains, and seeds in specified cultivation regions. This form of biofuel is unlikely to be a long-term feasible source of fuel because its production needs cultivated area, which, in turn, creates problems with feed/food use of feedstock (Hernandez *et al.,* 2021).

Alternatively, second-generation biofuels obtained from lignocellulosic biomass, like woody crops (sugar cane baggase, rice straw, corn cob, cotton stalk and wheat straw) residues are several viable options in terms of lower prices, output/input energy ratio and high availability (Shrama *et al*., 2020 and Barakat *et al*., 2016). Biofuels can be added in various proportions to gasoline are utilized in particular conditioned motors as pure biofuels. Moreover, as compared to fossil fuel, bioethanol is an outstanding fuel for future hybrid engines because it has a clean combustion (Duarte *et al*., 2021). It is also a biodegradable and oxygenated fuel (35% O_2) with cheap particulate matter and NOx content (Rahman *et al*., 2021). Bioethanol is expected to reach 140 billion litres in the global market by 2022 (Sharma *et al*., 2020).

Ethanol is a unique synthetic oxygen-containing chemical. Owing to its outstanding ability it has a few possessions like fuel, germicide, a depressant, antifreeze, a beverage, and as a solvent. Because of its dynamic nature, ethanol is a colorless, clear liquid, unstable and flammable.

For conventional consumption, bioethanol has a lot of benefits. An advantage of biomass-based biofuel is that it emits fewer emissions because fuel crops absorb CO_2 (Tyson *et al*., 1993). The rural economy benefits from the increased use of bioethanol as a result of growing such crops. Bioethanol is more environmentally friendly and less hazardous than fossil fuels. To fulfill energy needs bioethanol production from wastes products plays a major role (Wyman, 1994).

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The bacteria that ferment the ethanol in the absence of oxygen are known as anaerobic bacteria. Mostly ethanol is produced anaerobically. In the same way, fungi such as club fungus (*Basidiomycota*), yeast and sac fungi (*Ascomycota*), bread mould (*Zygomycota*), and chutrids (*Chytrimycota*) are utilized in the manufacturing of ethanol. The yeast *Saccharomyces cerevisiae* is the most commonly utilized fungus. For the production of ethanol from apples waste extract *Saccharomyces cerevisiae* plays an important role (Azhar *et al*., 2017 and Dias *et al*., 2015).

The important characteristic of this yeast is providing aroma and flavor to the various liquid refreshment and the significant task of yeast is in fermentation. *Saccharomyces cerevisiae* pure cultures are often employed in large-scale beverage manufacturing, such as distilled spirits, whiskey and brewing (Chen *et al*., 2017).

Ethanol production is obtained from a variety of sources. Glucose molecules are broken down during the fermentation process and then converted into ethanol by applying *S. cerevisiae* culture. Sugars from sugar cane, molasses, and sugar beets, starch from grains, wheat, maize and cellulose, which is usually utilized as raw material, are examples of glucose molecules. The main five sugars containing six-carbon sugars include xylose, arabinose, galactose, and mannose (Wyman, 1996). *S. cerevisiae* at a small scale grows at a temperature of about 20ºC to 30ºC and pH is 4.5-6.5. Apples belong to the Malus genus and Rosacea family. This is a broad family that includes not just apples but also palms, raspberries, peaches, and pears, among other fruits (Zhao *et al*., 2016).

In Pakistan, 13 lac 35 thousand tons of apples are produced each year. Pakistan is the tenth-largest producer of apples in the world. France, America, Italy, and Chile are all well-known for their apple production. Every year, they produce 50 million tons of apples. Baluchistan province is the first contributor of apple production whereas KPK is the 2nd contributor in Pakistan. About 25% of the apples are produced in the KPK province. The season for apple production runs from December until the end of January (Muhammad *et al*., 2011 and Noonari *et al*., 2015).

In the event of an energy crisis, the current research is essential. The goal of this study was to use apples to make ethanol, which could then be utilized for a variety of reasons. Apples contain a large number of natural sugars. A large quantity of apples is being discarded annually because of the low quality in different ways, though these apples contain sugar contents. These sugar contents are present in apple wastes which can be used for ethanol production by using *S. cerevisiae* yeast.

MATERIALS AND METHODS

Selection of Apples and Yeast

Low-quality apples and yeast *Saccharomyces cerevisiae* were selected for ethanol production. Apples were purchased from the local market while the *Saccharomyces cerevisiae* was obtained from the culture bank of Govt. College University, Lahore Pakistan. YPD medium (Yeast extract 1%, Tryptophan 0.033%, Peptone 2%, Dextrose 2%) was used for the culturing of *S. cerevisiae*. In a biosafety cabinet, a single colony of S. cerevisiae was streaked on a YPD-agar plate and incubated overnight at 30°C.

Preparation of Inoculum

The overnight growing culture of *S. cerevisiae* was used to make a fresh inoculum. A single colony of *S. cerevisiae* was aseptically placed into newly made YPD broth and incubated at 30°C until its optical density reached 0.8 at 600 nm.

Fermentation

For ethanol production, the surface culture fermentation technology was used, and the following procedures were followed.

Apple Extract Preparation

500 kg of apples were weighed to make apple extract, which was made by blending them with 1000 ml of distilled water. A homogeneous mixture of apples was made. Apple's juice was filtered with muslin cloth and residue was again ground and extract was collected. After examining the total sugar contents, mixture was sterilized by autoclave. It was then utilized for inoculation of S. *cerevisiae* yeast after chilling.

Inoculation of *S. cerevisiae*

Under an aseptic environment, a freshly made inoculum of *S. cerevisiae* yeast was placed into a sterilized mixture of apples. In an incubator, flasks were cotton plugged and incubated at 30°C.

Determination of Ethanol and Sugar Contents

Qualitative measurement of the production of ethanol was performed at times after 24, 48, 72,

96 and 120 hours by using an alcoholmeter whereas the quantitative measurement was performed by dichromate method. Reduction in sugar contents was also determined by refractometer and by DNS method (Miller, 1959) after every 24 hours until all sugar contents were consumed.

Ethanol Production Optimization

The following factors were used to maximize the production of ethanol from low-quality apples:

Effect of Incubation Time on Ethanol Production

After inoculation of *S. cerevisiae,* the apple extract was incubated for different periods ranging from 24 to 120 hours at 30°C to evaluate the effect of incubation duration on ethanol formation. The ethanol production and reduction of sugar contents were determined by standard methods as mentioned above.

Effect of Incubation Temperature

Effect of incubation temperature was determined by applying different incubation temperatures ranging from 20-40°C. Ethanol production and sugar utilization was calculated after 72 hours by the DNS method and dichromate method.

Effect of pH on the Production of Ethanol

The effect of pH was examined by varying the pH of the apple extract from 2 to 7. After inoculation with *S. cerevisiae* all flasks were incubated at 30°C for 72 hours and then sugar consumption and ethanol production were calculated by standard methods.

Effect of Inoculum Size on Ethanol Production

To find out the effect of inoculum size, a range of inoculum sizes was used (1-5%) for the inoculation of apple extract. Incubation was performed at 30°C for 72 hours. Production of ethanol and sugar contents utilization was calculated by using standard methods.

RESULTS

Culturing of *S. cerevisiae*

To obtain a fresh culture, a yeast strain of *S. cerevisiae* was grown on an YPD-agar medium and incubated at 30°C overnight (Fig.1).

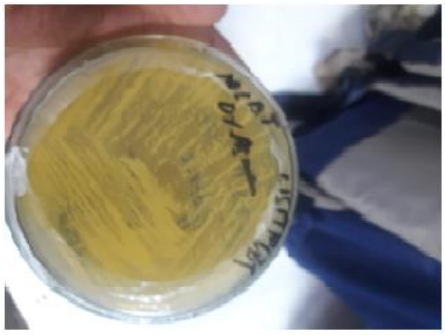

Fig.1: *Saccahromyces cerevisae* grown on YPD agar medium

Production of Ethanol from Low-quality Apples

Apple extract was prepared, sterilized and inoculated with *S. cerevisiae* for ethanol production. Following parameters were optimized for the maximum production of ethanol apples:

Effect of Time of Incubation

To check the effect of incubation time on ethanol production, the results showed that maximum ethanol (13%) was produced after 72 hours of incubation with minimum sugar contents (0.24%) as revealed (Fig. 2A). However, at other incubation times like 24, 48, 96 and 120 hours, the production of ethanol was 9.40%, 12%, 9.73%, and 7% with sugar contents of about 8.23%, 3.93%, 0.21%, 0.14%, respectively (Fig. 2A).

Effect of Temperature

Inoculated apple extract with an initial sugar concentration of roughly 20% was incubated at 20°C, 25°C, 30°C, 35°C, 37°C, and 40°C to observe the effect of temperature on ethanol formation using low-quality apples. The calculations were performed after 72 hours of incubation. As shown (Fig. 2B), maximal ethanol (13.23 percent) was produced at 30°C with minimal sugar concentrations (0.67 percent). At other temperatures like 25°C, 30°C, 35°C, 37°C and 40°C, ethanol production was 4.39%, 9.52%, 8.20%, 7.2% and 5% with sugar remnants as 10.75%, 4.62%, 6.53%, 8.03% and 10.42%, respectively (Fig. 2B).

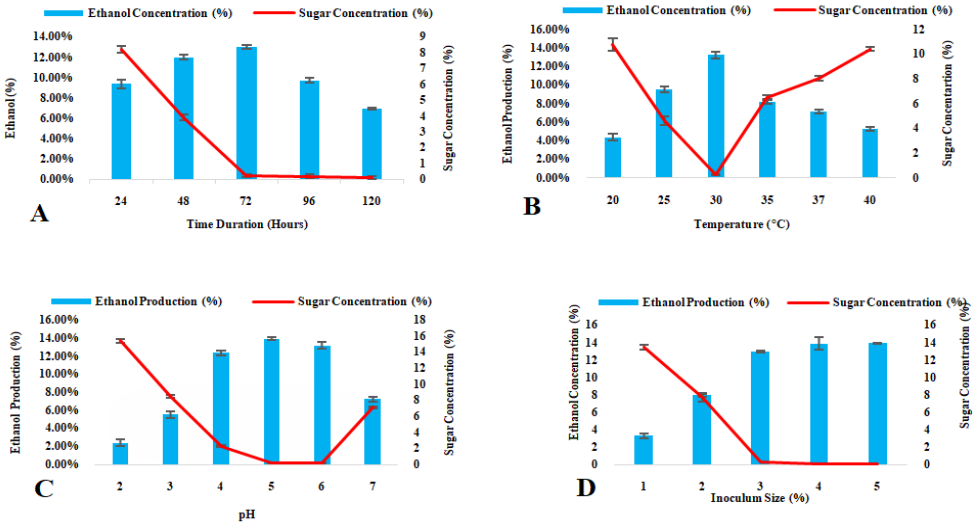

Fig. 2A, B, C, D: Effect of Time duration (hours), Temperature (°C), pH and Inocumum size (%) on ethanol production respectively.

Effect of pH

A pH range of 2.0 – 7.0 was used for the raw apple extract to test the effect of pH on ethanol production from low-quality apples using *S.cerevisiae*, and ethanol production was observed at 30°C after 72 hours of incubation. Results showed that the maximum ethanol (13.96%) was produced when the pH value of low-quality apple extract was 5.0 (Fig. 2C). The sugar contents at pH 5.0 were about 0.20%. At other pH values like 2.0, 3.0, 4.0, 6.0 and 7.0, values of ethanol production was 2.4%, 5.53%, 12.38%, 13.0% and 7.27% having sugar concentrations of about 15.47%, 8.58%, 2.28%, 0.23% and 7.13%, respectively (Fig. 2C).

Effect of Different Inoculum Size

To determine the inoculum size effect on ethanol production, the apple extract was inoculated with different yeast inoculums like, 1%, 2%, 3% 4% and 5%. The calculations were performed at 30˚C after 72 hours of incubation. Results showed that maximum ethanol (13.9%) was produced when 4% inoculum of *S. cerevisiae* was used with minimum sugar concentration (0.14%). However, at some other inoculum sizes like 1%, 2%, 3% and 5%, the ethanol was 3.32%, 8.03%, 12.98%, and 13.94% with sugar contents of about 13.21%, 7.69%, 0.29% and 0.15%, respectively (Fig. 2D).

DISCUSSION

Ethanol is a biofuel that can be produced by the fermentation of sugars by using *S. cerevisiae* culture. In the present study ethanol production was obtained from low-quality apple extract purchased from the local market of Lahore, Pakistan, by utilization of *S. cerevisiae*. The results showed in this work proved it has excellent potential for residue-based ethanol production. Moreover, ethanol production from the apples extracts can be observed under the concept of local market view. This research work is based on waste products of apples that were converted into ethanol. These extracts of apples contain sufficient sugar concentration which can be transformed into ethanol. The initial concentration of sugar contents is greater than reported by Singh *et al*., 2020.

Different factors were optimized for the maximum production of ethanol from low-quality apples by yeast *S. cerevisiae* including the effect of temperature, pH, inoculums size and time of incubation. An important factor that affects ethanol production is time duration. For the transformation of apples extract contents into alcohol contents, time period is important. The growth of *S. cerevisiae* regularly increased as the fermentation time duration increased. As a result, the fermentation process to a standstill, with no significant growth observable. A low ethanol product is obtained after 120 hours and maximum production was obtained after 72 hours (Fig. 2A). Sun-Waterhouse *et al.,* 2013 obtained the maximum ethanol production value after 48 hours of incubation. Our findings are consistent with those

reported in prior research utilizing apples extract. (Vucurovic *et al*., 2012 and Razmovski *et al*., 2012).

For ethanol production, the temperature of the incubator is a vital factor. We got the maximum production at 20ºC. In other research works Singh *et al*., 2014 obtained the minimum production at 25°C. At 30°C ethanol yield was most efficient and calculated as 10% as described in figure 2A. Our results are quite similar to Dominguez-Bocanegra *et al*. (2015) who produced ethanol from agricultural waste after the growth of yeast at 20ºC. Bellissimi & Ingledew (2005) found that ethanol is produced 30–33 times quicker when yeasts are in their log phase. The optimal temperature for *S. cerevisiae* is 20°C and maximum ethanol concentration was obtained. This could explain why ethanol fermented at 20 °C had a high sensory score due to its pleasant flavour and general acceptance. Various researchers have suggested that the final concentration of isobutyl alcohol, ethyl acetate, and isopentyl alcohol in fruit extracts ethanol production increases noticeably with an increase in fermentation temperature from 17 to 20 °C, and then decreases when the temperature is increased from 20 to 26 °C (Liu, 2017 and Yu *et al.,* 2003).

Essentially pH has an important role in ethanol production. Maximum ethanol production was obtained at pH 5.0 as shown in fig 2B. Low and high pH resulted in the inhibition of yeast growth which intern affected the ethanol yield. Yadav *et al*. (1997) obtained the maximum ethanol production at pH 6.0. Our findings are consistent with a prior study, which found that the ideal pH range for ethanol synthesis was 2.5-3.8 (Lin *et al*., 2012). This could be partially explained by the low pH, which improved yeast stability and reduced off-flavour.

Inoculum size had an impact on ethanol production. If the size of the inoculum is increased then ethanol production is also increased. In the fermentation process of ethanol, the inoculum size has great importance. There is a direct relation between inoculum size and the yield of ethanol. The quantity of ethanol is regularly increased if the size of the inoculum is increased. But the maximum amount of ethanol production of about 15% was obtained using an inoculum size of about 4%. However, if the size of the inoculum is increased, no considerable increase in ethanol production is observed. Our finding is in agreement with other works like Bajaj *et al*., 2001; Nowak, 2001; Kordowska-Wiater *et al*., 2001 and Alegre *et al*., 2003.

CONCLUSION

In the event of an energy crisis, the current research is important. The goal of this study was to find a way to use low-quality apples to make ethanol, which may then be utilized for a variety of applications. It is evident from this research that apples may produce the maximum amount of ethanol. In this study, maximum ethanol production was achieved from low- quality apples collected from markets of Lahore. *S. cerevisiae* strain was used for the maximum conversion of sugar contents of apple extract into ethanol. Different factors were optimized to get the maximum ethanol production that can be improved by further studies and could be beneficial candidates for various applications.

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