Nigerian Research Journal of Engineering and Environmental Sciences 2(1) 2017 pp. 153-160

Nigerian Research Journal of Engineering and Environmental Sciences
Journal homepage: www.rjees.com

Original Research Article

PRODUCTION OF ETHANOL FROM CORN STARCH USING DIFFERENT YEAST STRAINS

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ARTICLE INFORMATION

Article history:
Received 23 March 2017
Revised 17 April 2017
Accepted 25 April 2017
Available online 01 June 2017

Keywords:
Strains
Ethanol production
Saccharomyces cerevisiae
Alcoholic content
Starchy materials

ABSTRACT

This study presents the comparison of ethanol produced from corn starch using Saccharomyces cerevisiae obtained from different sources. Saccharomyces cerevisiae (S) bought from the market place and Saccharomyces cerevisiae (B) obtained from a brewery in Nigeria were used separately and in combination (SB). The colony counts and biochemical characteristics were determined. The parameters compared were alcohol content, specific gravity, pH and volume of alcohol produced. The temperature for production was between 25 – 30 ^{o}C and the pH range was between 4 and 7. The sample inoculated with the Saccharomyces cerevisiae (B) had the highest alcoholic content of 0.61% as against the combination of the different strains of Saccharomyces cerevisiae (SB) and Saccharomyces cerevisiae (S). Saccharomyces cerevisiae (B) produced the most alcohol with volume of 520 ml after the simple distillation processes were carried out.

1. INTRODUCTION

Recent years have seen the introduction of large scale processing in the bio-conversion of biomass resources especially starchy materials to ethanol which is expected to find a wide range of uses like bio-fuel and as starting material for various chemicals (Ghosall et al., 2013).

Ethanol is a volatile, flammable, colourless chemical compound. It is a monohydric primary alcohol and has a boiling point of 78.5 ^{o}C. It is miscible with water in all proportion (Gashaw, 2014). Ethanol production is a biological process which involves the conversion of sugars such as glucose, fructose and sucrose into cellular energy and thereby producing ethanol and carbon-dioxide as metabolic waste products (Gaur, 2006). Ethanol for use in alcoholic

beverages, and the vast majority of ethanol for use as fuel, is produced by fermentation. When certain species of yeast metabolize sugar in the absence of oxygen, they produce ethanol and carbon dioxide (Gasmalla et al., 2012).

Yeasts carry out ethanol fermentation on sugars in the absence of oxygen. They are a form of eukaryotic micro-organism classified in the kingdom fungi with about 1500 species described. (Rahman, 2013). The yeast, *Saccharomyces cerevisiae* has been used in baking and fermenting of alcoholic beverages for thousands of years (Belkin, 2010). *Saccharomyces cerevisiae*, the most common species of yeast, is used as both baker's and brewer's yeast. While the species of yeast is the same, different strains are often used to produce a sweeter taste in baked goods and a more bitter taste in brewed goods (Stewart, 2016). Yeasts belonging to *Saccharomyces cerevisiae* have been the commonly used of the various ethanol producing micro-organisms (Barnett, 2003). The majority of yeast used in baking is of the same species used in alcoholic fermentation. The most ethanol-tolerant strain of yeast can survive in a medium of up to about 25% concentration of ethanol by volume (Kurtzman, 2006).

Ethanol is considered “renewable” because it is primarily obtained from the conversion of the sun’s energy into usable energy. Creation of ethanol starts with photosynthesis which causes the feedstock such as sugar cane or corn to grow. This feedstock is then processed into ethanol (Shah and Sen, 2011). Corn is a rich source of many food and industrial products, one of which is ethanol. In the United States, one of the main feedstock for the production of ethanol is corn starch. Approximately 2.8 gallons of ethanol are produced per bushel of corn (Bothast and Schlicher, 2005).

Ethanol in developing countries is produced mainly by fermentation of dilute molasses at ambient temperature of 25 ^{o}C – 35 ^{o}C employing *Saccharomyces cerevisiae* (Savvides et al., 2000). Cane molasses is a complex mixture that varies in composition according to geographical sources, agricultural practices and sugar mill operations.

This study was carried out to evaluate the production of ethanol using different yeasts strains in order to achieve increase in the volume produced.

2. MATERIALS AND METHODS

2.1. Preparation of Sample

Saccharomyces cerevisiae (Saf-Instant by S. J. Lesaffre, 59703 Marcq, France) was bought from a market in Uyo, Akwa Ibom State, Nigeria. *Saccharomyces cerevisiae* (Brewer’s yeast) was procured from a brewery in Lagos, Nigeria. The corn was purchased from Itam market in Uyo, Akwa Ibom State, Nigeria. It was milled and screened with particle sizes ranging from 400-600µm collected for the study. The corn flour was mixed with water, filtered and the pH and specific gravity were measured.

2.2. Colony Counts and Biochemical Characterization

The samples (yeast bought from the market and yeast bought from the brewery) were serially diluted. Using spread technique, an aliquot of each sample was subculture on appropriately labeled Sabouraud Dextrose Agar (SDA) plate and incubated at 28°C for 48 hours. A colony count was done and the results were recorded. Yeast colonies were repeatedly sub-cultured on fresh sterile SDA plates and the pure isolates were purified and identified using standard yeast identification technique (Mossel et al., 1995).

2.3. Hydrolysis

Hydrolysis was carried out using concentrated sulphuric acid. The acid was measured using a 50 ml measuring cylinder and 20 ml of the acid was used to hydrolyse the sample. The acid was added gradually to the sample. The sample was boiled in a stainless steel pot on an electric stove (model Everest ES-1020) for 45 minutes. The samples were drawn at 5 minutes intervals and checked for starch hydrolysis using iodine solution. During boiling, the sample was stirred continuously with a glass rod to avoid gelling (Oboh and Aluyor, 2006).

2.4. Neutralization

Neutralization was carried out using 0.5 M solution of sodium carbonate (Na_2CO_3). The sample was allowed to cool to 40 ^oC from 98 ^oC before neutralization. The specific gravity of the cooled sample was determined before neutralization and then the sample was neutralized using 420 ml of the 0.5 M Sodium trioxocarbonate (IV) solution.

2.5. Fermentation

The sample was divided into three parts (1.61 litres) each and transferred into different bottles marked; Sample S, Sample B and Sample SB. Ten grammes of the yeast bought from the market (S) were used. The yeast was activated by warming to about 37^oC and later used to inoculate the Sample S. Ten millilitres of the yeast from the brewery (B) already in liquid form was activated by warming up to 37^oC to get the yeast cells alive before inoculating the Sample B. Five grammes of the yeast bought from the market (S) and 5 ml of the yeast from the brewery (B) were mixed together, and used to inoculate the Sample SB. The sample S was fermented using the *Saccharomyces cerevisiae* bought from the market and labeled S, the Sample B was fermented with *Saccharomyces cerevisiae* from the brewery industry and labeled B while a third sample labeled SB was obtained by fermenting with a combination of both strains of *Saccharomyces cerevisiae*. Fermentation was carried out for 72 hours at room temperature under anaerobic conditions. The temperature tolerance of *Saccharomyces cerevisiae* is 40^oC (Torija et al., 2003). The optimal time of fermentation was 48 hours (Gaur, 2006). Two hundred and fifty millilitres of the samples were drawn after every 24 hours for analyses. At the end of the fermentation process, a centrifuge was used to separate the microorganisms and the samples were then refrigerated at low temperature to deactivate any yeast cells that survived the separation. Samples were then analysed for alcohol

concentration, volume of production, pH, and specific gravity using an Anion Paari-Alcolyzer Plus where necessary.

2.6. Distillation

After 72 hours, the samples were distilled using a simple distillation set. Cooling water was circulated through the condenser for cooling and thereby condense the alcohol vapour. Distilled products were stored in bottles and cocked firmly to prevent evaporation.

3. RESULTS AND DISCUSSION

Table 1 shows the microbial counts of *S. cerevisiae* yeast strains. Yeast B had the highest cell count of 10.7 x 10^6 cfu/ml. This is indicative of its viability (Yabaya and Jatau, 2009). Table 2 shows the biochemical characteristics of the two strains of *S. cerevisiae* studied. The performance of the yeast strains on the basis of markers has shown very strong acid and gas production. The fermentative abilities of the two yeast isolates are identical.

Table 1: Colony counts on SDA

Yeast	Yeast counts (cfu/ml)
S	9.1 x 10^6
B	10.7 x10^6

Table 2: Biochemical Characteristics of *S. cerevisiae* isolates

Yeast	Sugar Fermentation						Nitrate Assimilation		
Isolates	A	B	C	D	E	F	KNO_3	$(NH)_4SO_4$	Ascospore
S	AG	AG	-	AG	-	AG	-	+	+
B	AG	AG	-	AG	-	AG	-	+	+

Key: A = Glucose, B = sucrose, C = Galactose, D = Maltose, E = lactose, F = Raffinose, AG = Acid and Gas production, A = Acid production only, + = growth, - = No growth

After twenty-four hours of inoculation with the yeasts separately and in combination, results presented in Figure 1 shows that Sample B had the highest value of alcohol percentage of 0.12% as compared to 0.05% for Sample S and 0.08% for Sample SB. This could be as a result of a specialized yeast strain for fermentation. By the end of the second day, Sample SB had its percentage alcohol as 0.28%, with 0.222% for Sample B and 0.23% for Samples S. At the end of the third day, Sample B had percentage alcohol value of 0.61% while Sample S had value of 0.25% and Sample SB was 0.43% and this could be corroborated by literature (Oboh and Aluyor, 2006).

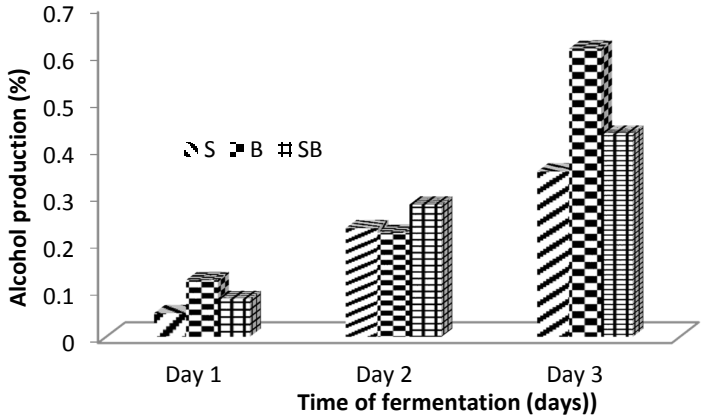

Figure 1: Variation of alcohol production with days of fermentation for different innoculum

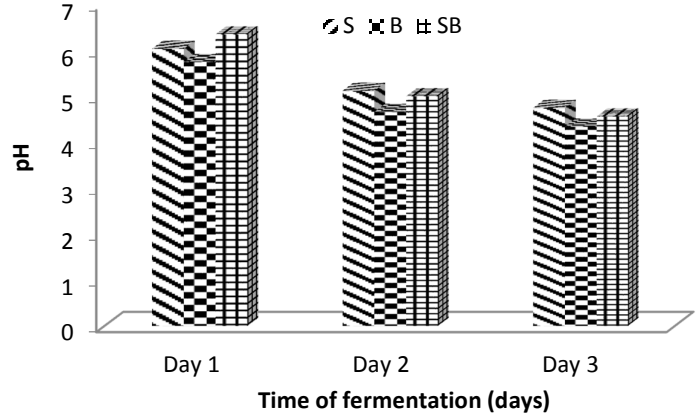

Figure 2: Variation of pH of samples with days of fermentation for different innoculum

The pH is a measure of the acidity or alkalinity of a solution. The initial pH of the samples was adjusted to 6.22 which was slightly acidic. Figure 2 showed that at the end of day one of fermentation, the pH of Sample SB was the least acidic with a value of 6.34 while Samples S and B followed with pH values of 6.03 and 5.74 respectively. During fermentation, alcohol was being produced and hence the systems became more acidic (Narendranath and Power, 2005). By the end of day two the samples became more acidic. Sample S with the pH value of 5.12 as compared to 4.66 for Sample B and 5.00 for Sample SB. Finally, by the end of the third day, the pH values further reduced to show that the samples were more acidic as more alcohol was formed as a result of fermentation. The pH values were 4.75 for Sample S, 4.43 for Sample B and 4.56 for Sample SB.

The specific gravity of the solution on the first day of fermentation was 1.0267 for Sample S, 1.0725 for Sample B and 1.0251 for Sample SB as shown in Figure 3. On the second day, specific gravity values were 1.0271 for Sample S, 1.0244 for B and 1.0261 for Sample SB. The specific gravity of samples showed a higher value on the second day of fermentation and

this could be as a result of increased alcohol production. Finally, on the third day, specific gravity further increased for the samples as alcohol percentage per sample increased. Sample B had the highest value of specific gravity on day three and this clearly showed why there was a high alcoholic concentration on the last day of fermentation.

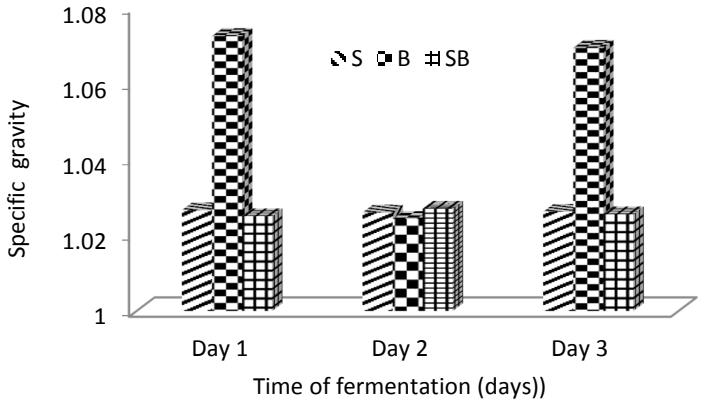

Figure 3: Variation of Specific gravity of samples with days of fermentation for different innoculum

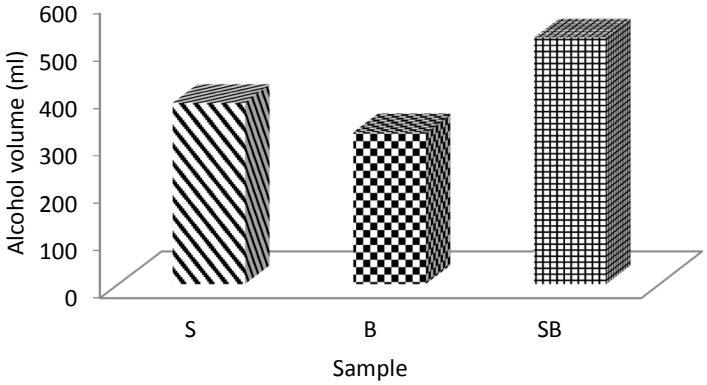

Figure 4: Volume produced for various yeast used after sample distillation

Each sample had an initial volume of 1.61 litres before inoculation with the various yeast. Figure 4 shows that after the three days of fermentation and simple distillation Sample SB had the highest volume of 520 ml as against 382 ml for Sample S and 320 ml for Sample B. Sample SB had the highest volume of alcohol because brewer's yeast and baker's yeast both turn sugars into alcohol and carbon dioxide. Both of these yeasts are made from strains of the Saccharomyces cerevisiae fungus, but each from different strains of this specie. Different strains of Saccharomyces cerevisiae produce different proportions of carbon dioxide and alcohol. Baker's yeast is a blend of several strains of Saccharomyces cerevisiae chosen for their flavor and ability to make carbon dioxide, which causes bread to rise. Brewer's yeast is

made of strains chosen for their alcohol-producing ability and tends to have a bitter flavour (Kalmus, 2014).

4. CONCLUSION

In this study, the performance of different yeasts separately and in combination on some parameters in ethanol production were investigated. The results obtained from this study showed strength for certain parameters when considered. Hence depending on the need, it would be possible to choose any of the yeast that would be effective in achieving the desired result. For this study it was found out that the sample with both strain gave the highest volume of ethanol followed by the sample with the brewer's yeast.

5. ACKNOWLEDGMENT

The authors wish to acknowledge the assistance and contributions of the laboratory staff of the Department of Chemical and Petroleum Engineering Department, University of Uyo, Uyo toward the success of this work.

6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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