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A Kinetic Study of Burukutu Fermentation

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Abstract: The yeast fermentation of malted sorghum for burukutu has been investigated by following the sugar depletion profile at 20, 25 and 30°C. Fermentations were carried out in a 2L laboratory fermentor at a constant pH of 3.5, while the sugar concentration was determined by the titration method of Lane and Eynon. A test of the Michaelis-Menten model for shifting-order kinetics and a second-order model for fit on the sugar depletion data, showed that while the Michaelis-Menten model failed to describe the mode of sugar consumption, the second-order model gave a good fit of the data for all the fermentation runs. The kinetics of the fermentation of burukutu beer therefore, follow a second-order model. The fermentation rates increased with temperature and were higher for seeding with yeast compared with the practice of using an old brew of burukutu. The rate constant ranged from 0.1070-0.1541 L $(cell)^{-1}hr^{-1}$ within the temperature range investigated, with activation energy of 71.89 KJ $Kmol^{-1}$. It would appear that the use of a starter culture of yeast could ensure higher fermentation rates, but the effect of this on the burukutu beer quality would need to be examined.

Key words: Beer brewing, burukutu, fermentation, malted sorghum, second-order model

INTRODUCTION

Fermented foods are an essential part of diets in all regions of the world. Sorghum, otherwise known as guinea corn is among the cereals which provide the bulk of diet for large population in the tropics. Two species of sorghum are common, sorghum bicolor and sorghum vulgare (Okorie and Oke, 2003).

Starch-containing cereals such as barley, sorghum, maize, millet, wheat etc, are the starting materials, which provide the sugars for beer brewing. Starch is not readily fermentable by yeast to alcohol, but must first be hydrolyzed to simple sugars by the process of malting. Some indigenous African beers made from malted sorghum include, burukutu, otika, borde, kaffir, bouza, pombe, shukutu, shakparo, amgba, dolo, tchapalo, kununzaki and pito. Because the ingredients and procedures for making these beers differ, the beer characteristics such as taste, color and nutritional value also vary.

Burukutu is an indigenous beer brewed at the cottage level in some parts of West Africa. The basic characteristics of burukutu include, a sour taste due to the presence of lactic acid, a pH of 3.3 to 3.5 and opaque color because of suspended solids and yeast. It contains vitamins, iron, manganese, magnesium, phosphorus and calcium and also contains about 26.7g of starch and 5.9 g of protein per litre. In traditional burukutu fermentation, the yeast used is cultured or maintained in an immobilized form on a fermenting vessel, but frequently, an old brew of burukutu is used as the source of the fermenting yeast.

Lactic acid bacteria especially the thermophilic lactobacilli have been identified as been responsible for the conversion of the starch to fermentable sugars in burukutu brewing (Ihekoronye and Ngoddy, 1985). In a fermenting mixture of burukutu, the organisms isolated are yeast-mainly Saccharomyces cerevisiae and S. chaveleri and the bacteria, Leuconostic mesenteriodes (Achi, 2005). Spontaneous fermentations are difficult to control, are not predictable in terms of length of fermentation and quality of product; can produce unwanted products or products with a short shelf life. To overcome these problems, the most predominant microorganism found in an acceptable product can be isolated, purified and used as starter culture to initiate the fermentation (Togo *et al*., 2002).

In burukutu brewing at the cottage level, process parameters such as temperature, pH and yeast addition are not optimized. The optimization of processing parameters for malting, mashing and fermentation are indispensable priorities for upgrading burukutu brewing to industrial or commercial scale (Abegaz *et al*., 2002). A kinetic study of the nature undertaken in this study therefore, holds promise for the possible upgrading of the African indigenous processes of burukutu brewing.

MATERIALS AND METHODS

Yeast: The yeast strain, Saccharomyces cerevisiae (brewers yeast) used in this work was obtained from the

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central laboratory at the National Root Crops Research Institute, Umudike in Abia state, Nigeria.

Sorghum: The red sorghum variety used for this research was obtained from local market in Imo state, Nigeria. Using the method of Lasekan and Lasekan (1997) the sorghum grains were steeped (1.5 Kg grains in 2L of water) for 24 h, air rested for 4 h. and germinated using an incubator (model E2A) at 25°C for 5 days. The grains were watered every morning and turned over at intervals of 24 h. Kilning was done at 55°C for 24 h. using a moisture extraction oven (model PF200), followed by milling with an Atotomus laboratory mill.

Old brew: The old brew of burukutu used for inoculation in this study was obtained from the open market in Owerri, Imo state.

Mashing: The mashing of the sorghum malt was carried out by a modified method of Igyor (2005). 4.0 Kg of sorghum malt was weighed with a weighing balance (model LC1201S) and mixed with 4.8L of warm water (45°C). The mixture was stirred and allowed to settle for 30 min. When settled, 2L of the clear enzymic supernatant was decanted and the remaining mash was gradually brought to boil at 100°C and held at the same temperature for 30 min. The mash was cooled to 60°C and the enzymic supernatant was added and then kept for 12 h for lactic acid fermentation which turned the brown color of the malt yellowish. The mixture was filtered through a sieve mesh (200μm) and rinsed with 300 mL of water (50°C) to extract the remaining enzymes from the grist. The wort was further boiled for 1½ h, cooled and the pH adjusted to 3.5 using acetic acid buffer.

Preparation of yeast for inoculation: One gram of the dry Saccharomyces cerevisiae was weighed with a balance (model LC1201S), added to 100 mL of the sorghum wort and was then left for 6 h. to activate the yeast strain.

Fermentation of sorghum wort: The inoculated sorghum wort was added to 400 mL of sorghum wort for the alcoholic fermentation. Fermentation was carried out in a 2L laboratory fermentor.

Three fermentation runs were carried out by seeding with external yeast at 20, 25 and 30°C, at a constant pH of 3.5. Ten mililiter of samples were taken from the fermentation broth at intervals of 1 h, for the determination of sugar concentration. Fermentation was also done by inoculating with an old brew of burukutu (sorghum beer). Hunderd mililiter of an old brew was added to 400 mL of the sorghum wort, in line with the traditional method of brewing burukutu. The fermentation was carried out at 30°C and a pH of 3.5. Ten mililiter samples were taken from the fermentation broth at 1 h. intervals for the determination of sugar concentration.

Determination of sugar concentration: Sugar concentration was determined by the Lane and Eynon method (ISI, 1999). Ten mililiter of mixed Fehlings solution was pipetted into a conical flask and 4 drops of 1% methylene blue was added. The solution was brought to boil. While boiling, a standard sugar solution (0.5 g of glucose in 100 mL of distilled water) was added from the burette until the blue color disappeared. The titration was repeated using sample solution instead of standard glucose solution. The concentration of sugar in sample was determined as

$$C_s = (C_g * a) / b \ (g\ mL^{-1})$$

Analysis of data: Two kinetic models were tested for fit on the sugar depletion data.

Model 1: The Michaelis-Menten model has been used widely to describe the kinetics of enzyme reactions of simple sugars. The rate equation for substrate depletion can be written as Eq. 1, which can be

$$\frac{-dS}{dt} = \frac{KS}{Km + S} \quad (1)$$

integrated to give Eq. 2;

$$\frac{\ln(S/SO)}{S - S_o} = \frac{Kt}{K_m\ (S_O - S)} - \frac{1}{K_m} \quad (2)$$

Hence a plot of $\ln (S/S_O)/S\text{-}S_O$ against $t/S_O\text{-}S$ gives a straight line with slope K/K_m and intercept of $-1/K_m$ (Ikhu-Omoregbe and Omolaiye, 1996).

Model 2: Second-order kinetic equations are the multiple-substrate removal kinetics commonly found in literature (Kovarova-Kovar and Egli, 1998). A second-order model of the form proposed by Paris *et al.* (1981) was used.

$$\frac{-dS}{dt} = K_2\ S\ X \quad (3)$$

Since experimental growth is the most encountered in liquid cultures (Brunner and Focht, 1984) then X can be expressed as a function of t as;

$$X = X_O\ e^{(\mu t)} \quad (4)$$

Substitution of Eq. 4 into 3 and integrating gives;

$$S = S_O e^{[-K_2 (X_O/\mu)(\exp \mu t-1)]} \quad (5)$$

Equation 5 is intrinsically non-linear, hence a non-linear regression, using the GraphPad Prism 4 software (G.S.I,1996) was used to fit the curve to the data. In non-linear regression, the initial estimates of the unknown parameters need not be accurate, but are required to start the iterative process, which then yields a convergence. An estimate of $0.29h^{-1}$ for μ was taken from literature values reported for yeast fermentation of glucose by McNeil and Harvey (1990). Using this value of μ, an initial estimate of K_2 was then determined from Eq. 5 by putting t = 4 h., to be 0.078.

RESULTS AND DISCUSSION

The results of the experimental runs are shown in the progress curve of Fig. 1. Regression plots to test for the

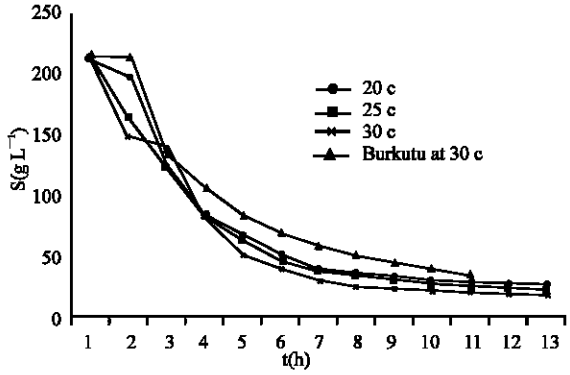

Fig. 1: Plot of substrats consumption

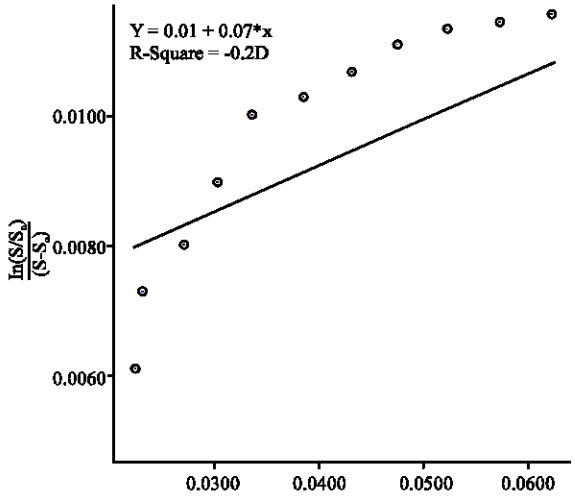

Fig. 2: Regression plot of Eq. 2 for external yeast at 20 °C

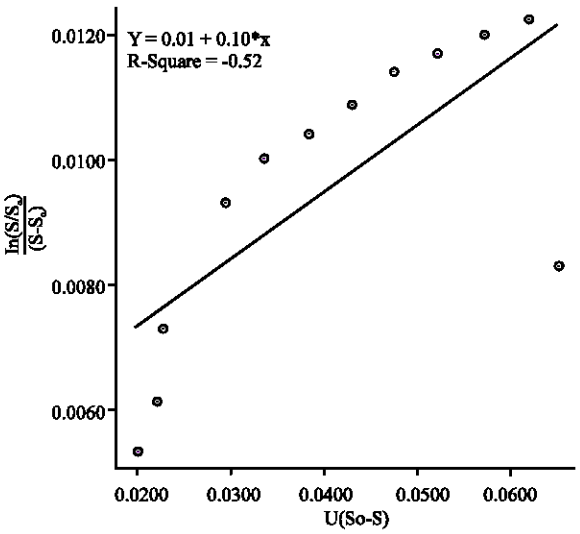

Fig. 3: Regression plot of Eq.2 for externaly yeast at 25 °C

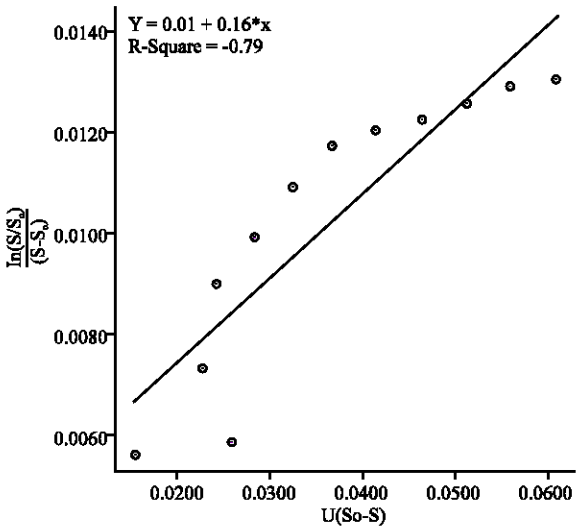

Fig. 4: Regression plot of Eq. 2 for external yeast at 30 °C

Michaelis-Menten model on the data are shown in Fig. 2-5, while the summary of constants from these plots are given in Table 1. Figure 6-9 are the curves of the non-linear regression of the second-order model, while the best fit values of the unknown parameters are displayed in Table 2.

Figure 1 reveals that the fermentation rate increased with temperature as expected. It is also evident from the progress curve that fermentation rates were higher for all cases of seeding with yeast, compared with inoculating with the old brew of burukutu, as is the practice in some of the traditional processes. This suggests that the use of

Table 1: Summary of constants from plots of the Michaelis-Menten model

Temp(°C)	K(hr^{-1})	K_m(g L^{-1})	R^2
20	-7	-100	0.20
25	-10	-100	0.52
30	0	0	0.79
30(old brew)	0	0	0.95

Table 2: Summary of parameters from non-linear regression plots of the Second-order model

Temp(°C)	$K_2L(cell)^{-1}hr^{-1}$	$\mu(hr^{-1})$	R^2
20	0.1323	0.030	0.96
25	0.1414	0.020	0.98
30	0.1541	0.016	0.98
30(old brew)	0.1070	0.038	0.95

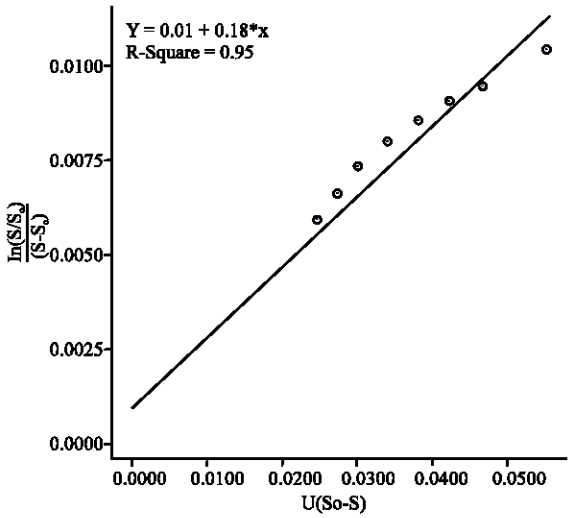

Fig. 5: Regression plot of Eq. 2 for old brew at 30°C

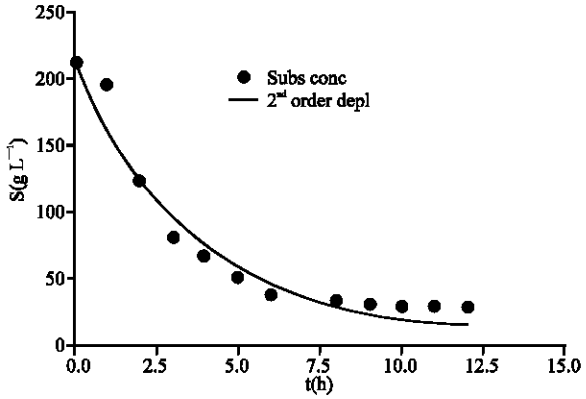

Fig. 6: Second order depletion of substrate using external yeast at 20°C

a starter culture of yeast would ensure better fermentation rates for burukutu brewing. However, the effect that this would have on the nutritional value, taste and other characteristics of the beer will need to be investigated.

A visual inspection of Fig. 2-5, show that the scatter points on the plots don't follow a linear model. The plots

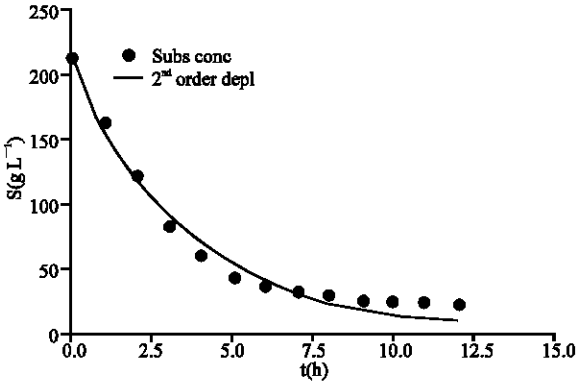

Fig. 7: Second order depletion of substrate using external yeast at 25°C

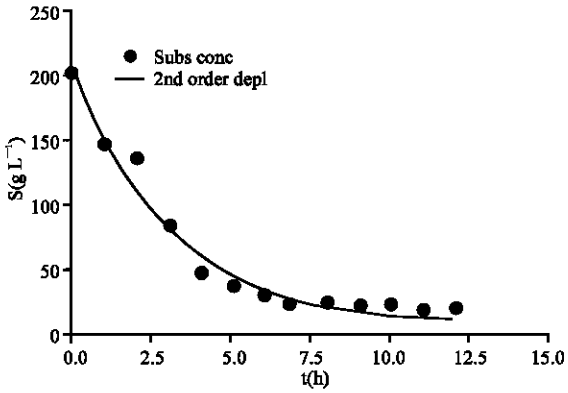

Fig. 8: Second order depletion of substrate using external yeast at 30°C

gave either negative or zero values for the Michaelis constant K_m and the rate constant K and also gave low R^2 values (Table 1). R^2 is a measure of the goodness of fit of the model to the data. These clearly indicate that the Michaelis-Menten model for shifting-order kinetics does not describe the mode of sugar utilization in the fermentation of malted sorghum for burukutu. The Michaelis-Menten kinetics was formulated on the basis of constant catalyzing material and is therefore applicable to a situation in which the microbial cells participating in the degradation are not growing to any significant degree (Kovarova-Kovar and Egli, 1998). Also, while the Michaelis-Menten model can describe many enzyme catalyzed reactions involving a single substrate, it does not apply to multiple-substrate systems as would be the case in a fermenting broth of malted sorghum which contains a mixture of fermentable sugars.

An inspection of Fig. 6-9, show that a second-order model of the form adopted is able to simulate the sugar utilization in burukutu fermentation. It gave a good fit of the data for all the fermentation runs, with high R^2

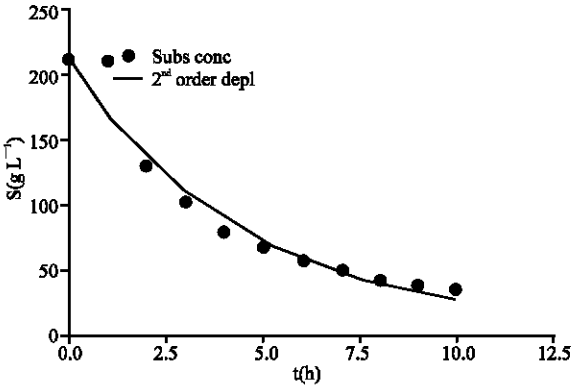

Fig. 9: Second order depletion of substrate using old brew to inoculate at 30°C

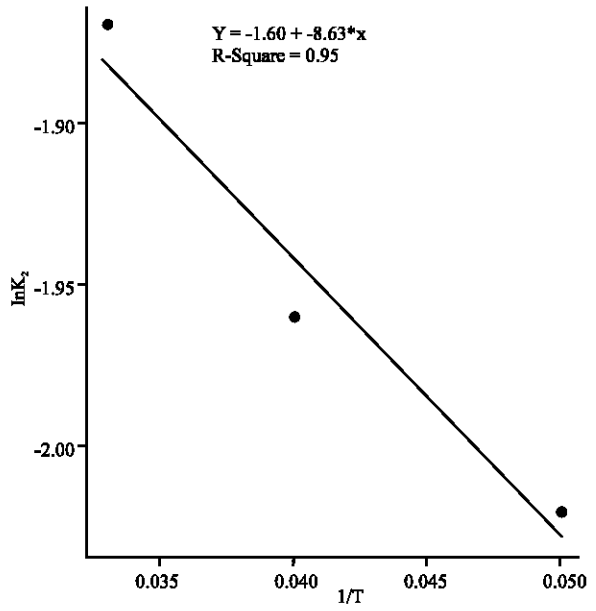

Fig. 10: Plot for evaluating E_a

values (Table 2). Second-order kinetic equations have been applied commonly in literature to multiple-substrate removal (Kovarova-Kovar and Egli, 1998; Paris *et al*., 1981).

The integrated form of the second-order model (Eq. 5) is simple and deterministic and the rate constant K_2 has an intrinsic meaning. The equation is also able to express substrate concentration as an explicit function of time. The rate constant K_2 increased with temperature and was higher for all cases of seeding with yeast compared with using an old brew of burukutu. The graph of $\ln K_2$ against 1/T was plotted as shown in Fig. 10.

By the Arrhenius equation, the slope of this line represents $(-E_a/R)$. E_a was determined to be 71.89 KJ $Kmol^{-1}$ from the plot.

CONCLUSION

The kinetics of sugar depletion in burukutu fermentation, follow a second-order model of the form

$$\frac{-dS}{dt} = K_2 \, S \, X$$

This agrees with the applicability of second-order kinetics to multiple substrate removal in systems containing a mixture of substrates like a fermenting broth of malted sorghum, as commonly found in literature.

The reaction rate increased with temperature and was higher for seeding with yeast compared with an old brew of burukutu. At a pH of 3.5, the rate constant ranged from 0.1070-0.1541 L $(cell)^{-1}$ h^{-1} within the temperature range investigated, while the activation energy was 71.89 KJ $Kmol^{-1}$.

Notation:

- a mL standard glucose solution for titration
- b mL sample for titration
- C_g Concentration of standard glucose solution (g mL^{-1})
- C_s Concentration of sugar in sample (g mL^{-1})
- E_a Activation energy (KJ $Kmol^{-1}$)
- K Rate constant (hr^{-1})
- K_m Michaelis constant
- K_2 Second-order rate constant (L $(cell)^{-1}hr^{-1}$)
- R Gas constant (KJ $Kmol^{-1}$ K)
- S Sugar concentration (g L^{-1})
- S_o Initial sugar concentration (g L^{-1})
- T - Temperature
- X - Concentration of yeast (g L^{-1})
- X_o - Initial concentration of yeast (g L^{-1})
- μ - Specific growth rate (hr^{-1})

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