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Kinetic Modelling of Ethanol Inhibition during Alcohol fermentation of Corn Stover using Saccharomyces Cerevisiae

[*]Amenaghawon, N.A, Okieimen, C.O and Ogbeide, S.E
[*]Department of Chemical Engineering
Faculty of Engineering
University of Benin, PMB 1154, Benin City, Nigeria

ABSTRACT

The inhibitive effect of ethanol on the growth of fermenting organism during batch ethanol fermentation was investigated. A low order kinetic growth model was adopted to simulate cell growth. Experimental data was used to test the validity of the kinetic model. Results show that only the Hinshelwood model was able to replicate to a high level of confidence, the concentrations of substrate, biomass and ethanol as obtained experimentally. Results obtained by using kinetic parameters estimated by the Hinshelwood model to simulate cell growth showed that cell yield, product yield, specific growth rate and specific ethanol production rate were all affected by ethanol inhibition. The inhibitory effect of ethanol on the specific growth rate, product yield and specific ethanol production rate was observed to be primarily due to decreasing biomass yield.

Keywords - Kinetics, cell growth, Saccharomyces cerevisiae, Hinshelwood model, Ethanol fermentation

1. INTRODUCTION

In order to effectively analyse and subsequently optimise a biological process, the kinetics of the process needs to be understood and quantified [1]. The use of kinetic models to describe the behaviour of biological systems has been acknowledged to be important because it can reduce the number of experiments needed to eliminate extreme possibilities and provide mathematical expressions that can quantitatively describe the mechanism of the process as required for optimization and control [2], [3]. Though a lot of kinetic models have been developed for the growth of cells in both batch and continuous processes, unstructured models still give the most basic understanding of metabolism of microbiological processes [4–6]. These unstructured models fairly approximate the dynamic behaviour of these processes for non-steady state cases [7].

The growth of microorganisms during bioconversion is a complex process. The Monod equation is usually used to relate the specific growth rate to the concentration of the limiting substrate.

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad (1)$$

This equation is well suited for fermentation processes where the growth of fermenting organism is not inhibited by toxic substances.

It is known that microbial activity during ethanol fermentation is affected by certain factors namely, cell death, substrate limitation, substrate inhibition and product inhibition. . None of the models in the literature account for the effect of these factors at the same time. The models of Egamberdiev & Jerusalimsky [8], Ghose & Tyagi [9], Hinshelwood [10], Holzberg et al. [11], Hoppe & Hansford [12], Lee [13] and Aiba et al. [14] only account for the effect of product (ethanol) inhibition. For a kinetic model to appropriately represent microbial activity during ethanol fermentation, it should account for the effect of all four factors. Even though this is the desired outcome, it is not realistic to expect that any kinetic model to will be able to correctly represent real process situations.

The purpose of this study is to investigate the biokinetics of batch ethanol fermentation. By using a kinetic model that accounts for ethanol inhibition, this paper examines the inhibitory effect of ethanol on cell growth and ethanol productivity during fermentation. Model parameters were estimated using experimental data and these were subsequently used for computer simulations to predict how the ethanol concentration affected cell yield, ethanol yield, cell growth rate and ethanol production rate.

2. MATERIALS AND METHODS

2.1 Materials collection

Corn stover was obtained from a farm in the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria. The yeast

Saccharomyces cerevisiae ATCC 4126 which was obtained from Bendel Brewery Nig. Ltd., Benin City, Nigeria was used as fermenting organism in this study.

2.2 Culture medium, inoculum and fermentation

The composition (g/L) of the fermentation medium used for ethanol production was: Glucose, 100; Yeast extract, 8.5; NH_4Cl, 1.32; $MgSO_4.7H_2O$, 0.11; $CaCl_2$, 0.06; Antifoam, 0.01mL; Citric acid, 1.5; Citrate, 0.2; Water, 1L. The fermentation was carried out in 1L Erlenmeyer flasks. The fermenting vessel was tightly corked to ensure anaerobic condition prevailed during the fermentation.

2.3 Substrate and pretreatment

The collected corn stover was sun dried to reduce its moisture content. The dried corn stover was milled into small particles to increase its surface area and make the cellulose readily available for hydrolysis. It was subsequently screened using standard sieves of known mesh sizes to obtain 0.6mm particles. Dilute acid hydrolysis of the corn stover was carried out using 250ml of 1% sulphuric acid at 190^{o}C for 2hours. Each hydrolysate was subsequently neutralised with 1.0M Sodium Hydroxide solution and then allowed to cool at room temperature. The neutralised hydrolysate was centrifuged for 20 minutes to remove any suspended solids [15]. Enzymatic hydrolysis was carried out by heating the neutralised hydrolysate at 100 ^{o}C for 10 minutes to gelatinise any starch present. Enzymatic hydrolysis was carried out with the aid of α and β amylase. At the end of hydrolysis, the enzymes were inactivated by heating at 100 ^{o}C for 10 minutes [16].

2.4 Analyses

Liquid samples were taken at intervals of 2 hours and analysed for glucose, biomass, and ethanol. Cell concentration was measured by dispensing 5 cm^3 of fermentation broth into a tube and centrifuging it at 5000 rpm for 30 minutes. The optical density of the sample was measured spectrophotometrically at 600nm and compared to standard curve of dry weight of yeast cells. The glucose content of the sample was determined using the DNS method [17]. The ethanol concentration was measured by the dichromate oxidation method, which is based on the complete oxidation of ethanol by dichromate in the presence of sulphuric acid [18].

3. RESULTS AND DISCUSSION

3.1 Cell growth kinetics:

The kinetic model developed by Hinshelwood [10] was adopted for describing cell growth during fermentation.

$$\mu = \mu_{\max} \frac{S}{K_s + S}\left(1 - \frac{P}{P_m}\right) \quad (2)$$

Where $\mu\,(h^{-1})$ is the specific growth rate, $\mu_{\max}\,(h^{-1})$ is the maximum specific growth rate of biomass, $K_s\,(g/L)$ is the substrate affinity constant, and P_m (g/L) is the maximum concentration of ethanol above which cell growth ceases.

3.2 Model validation and parameter estimation:

The adopted kinetic model was validated against experimental data by estimating the model parameters. Table 1 shows the parameters estimated and their respective optimal estimate.

Table 1: Values of estimated parameters

Model Parameter	Optimal estimate
$\mu_{\max}\,(h^{-1})$	0.36
$K_s\,(g/L)$	5.42
$P_m\,(g/L)$	30.13

These parameters were used to generate time profiles of substrate, biomass and product concentrations for the model. Fig. 1 shows the comparison between experimental data and the model predicted data for substrate, biomass and ethanol concentrations.

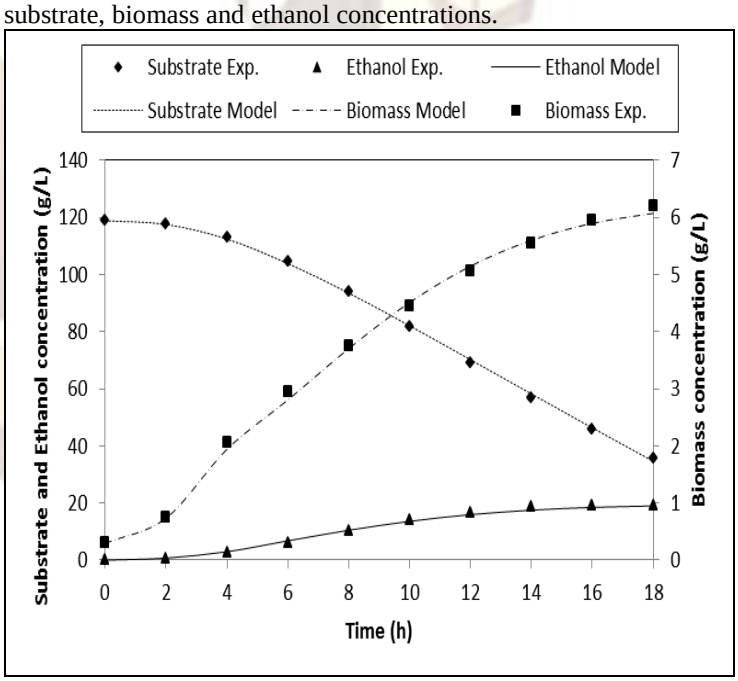

Figure 1: Comparison between experimental data and the model predicted data for substrate, biomass and product concentrations

The results presented in Fig 1 shows that the kinetic model adopted was able to replicate the concentrations of ethanol, substrate, and biomass as obtained from experiment. This is evident in the high level of correlation between the experimental and model predicted results, hence the model exhibits a good fit with the experimental data. There was a progressive decrease in the concentration of substrate with time. The observed trend indicates that the substrate is being metabolised by the fermenting microorganism to produce ethanol. This observation is mirrored by the corresponding progressive increase in the concentrations of ethanol and biomass since the cells metabolise the substrate to induce growth and subsequently produce ethanol. These results are in agreement with those reported by Baei et al. [19] and Ocloo & Ayernor [20]. According to them, they observed similar decrease in the substrate concentration as a result of ethanol formation by the metabolic consumption of the substrate.

3.3 Ethanol inhibition

In the following section, the inhibitory effect of ethanol is evaluated. This was accomplished by determining the effect of ethanol concentration of key fermentation variables. The parameters estimated were used to run simulations to evaluate the inhibitory effect of ethanol.

3.3.1 Biomass yield

Fig. 2 shows the effect of ethanol concentration on the yield of fermenting organism. It can be observed that, cell yield decreases gradually but progressively as the concentration of ethanol increases indicating a relationship between biomass yield and product inhibition.

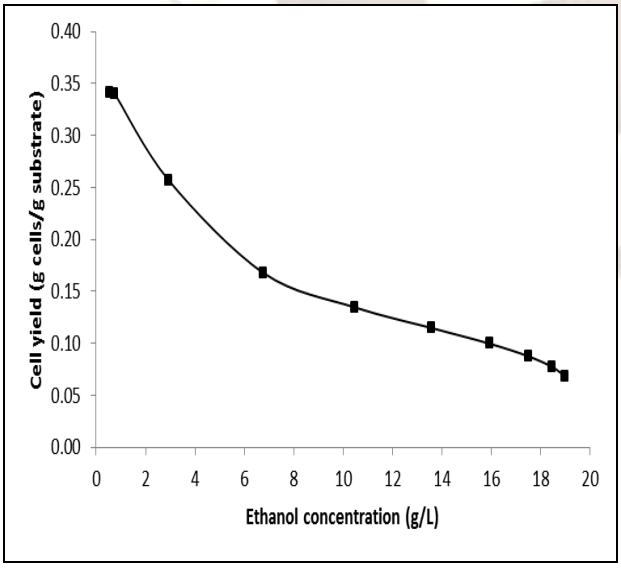

Figure 2: Effect of ethanol concentration on the yield of fermenting organism as predicted by the hyperbolic and Hinshelwood models

The decrease observed may be as a result of the accumulation of ethanol in the fermentation broth which inhibits the growth of the fermenting organism. Warren et al. [1] and Taylor et al. [21] reported similar declines in biomass yield with increase in ethanol concentration.

3.3.2 Ethanol yield

The ethanol yield like the cell yield, are assumed to be constant. In some cases, parameters representing cell death and maintenance are utilised to account for changes in cell and product yields. The effect of ethanol concentration on ethanol yield is illustrated in Fig. 3. It can be observed that the yield of ethanol increases as its concentration increases after which it becomes somewhat constant. The trend observed is easily explained in that as the concentration of ethanol in the fermentation vessel increases, the yield will also increase since yield is an indication of the amount of a substance produced. The yield becomes constant towards the end of the plot because inhibition has set in and the cells are no longer producing fresh ethanol. These results agree with those obtained by Warren et al. [1] where the characteristic plot of the yields as a function of ethanol concentration was similar to that presented here.

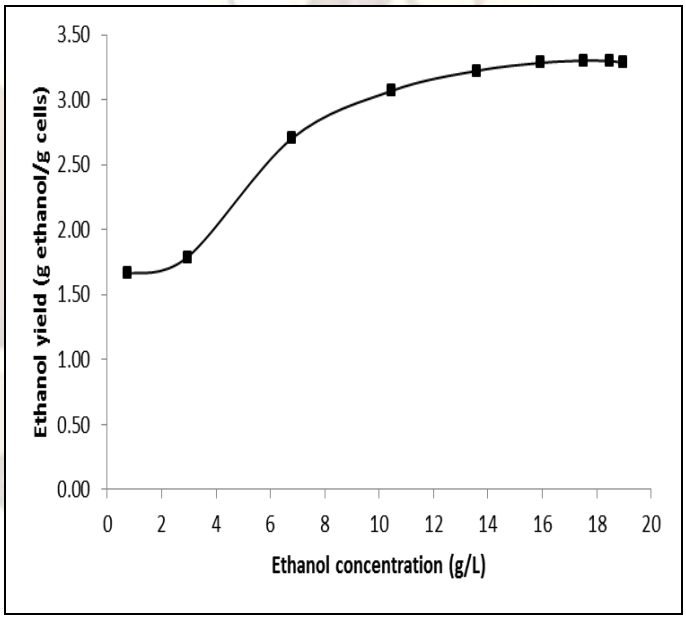

Figure 3: Effect of ethanol concentration on the yield of ethanol as predicted by the hyperbolic and Hinshelwood models

3.3.3 Specific growth rate

The inhibitory effect of ethanol on the specific growth rate as predicted by the hyperbolic model is shown in

Fig. 4. This was done by comparing the specific growth rate with its maximum value (μ_{max}).

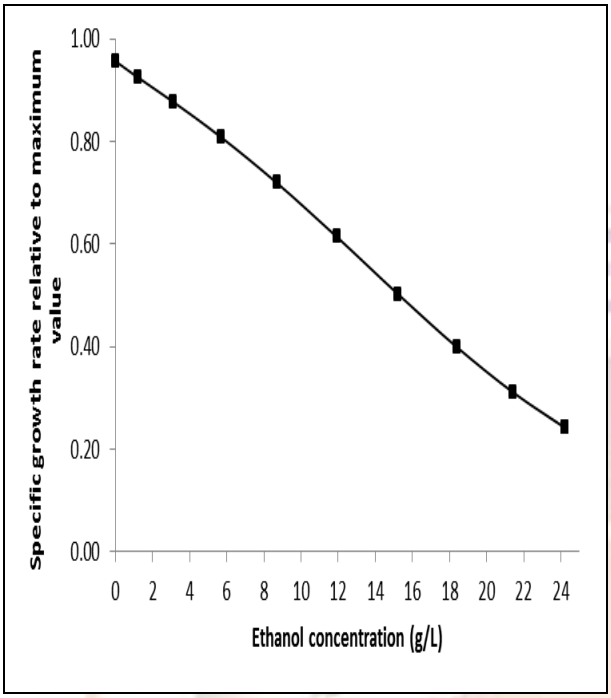

Figure 4: Effect of ethanol concentration on the specific growth rate as predicted by the hyperbolic and Hinshelwood models

It is observed from Fig. 4 that the model predicts an almost linear relationship between the relative specific growth rate and ethanol concentration. A steady decrease in the specific growth rate of fermenting organism relative to its maximum value is observed as the concentration of ethanol increases. The almost linear behaviour predicted by the Hinshelwood model is similar to those obtained by previous workers [11], [14]. The results clearly show that the inhibitory effect of ethanol on the specific growth rate is principally due to decreasing biomass yield as shown in Fig. 2.

3.3.4 Specific ethanol productivity rate

Fig. 5 is a plot of the specific rate of ethanol production as a function of ethanol concentration. The model predicts that the specific ethanol productivity rate decrease steadily as ethanol concentration increases. The Hinshelwood model predicts a linear relationship between specific ethanol productivity rate and ethanol concentration. These results are similar to those obtained by Daugulis & Swaine [22].

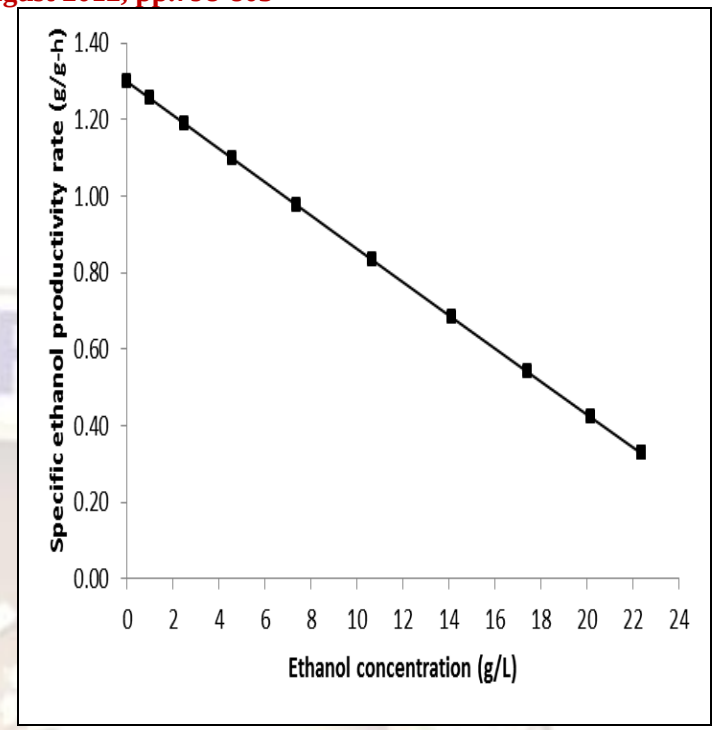

Figure 5: Effect of ethanol concentration on the specific ethanol productivity rate as predicted by the hyperbolic and Hinshelwood models

4. CONCLUSIONS

In this work, the effect of ethanol inhibition during batch fermentation of ethanol from pretreated corn stover using *Saccharomyces cerevisiae* has been investigated. A low order kinetic model was adopted to simulate cell growth. The following conclusions can be drawn from this study.

- The validated Hinshelwood model which is a kinetic growth model that account for product inhibition can predict the dynamic response of cell growth during ethanol fermentation. This was evident in the high level of correlation between the experimental results and the model predicted results.
- A linear relationship exists between relative specific growth rate and ethanol concentration. A linear relationship also exists between specific ethanol productivity rate and ethanol concentration.
- The primary effect of ethanol inhibition is on biomass yield which in turn affects other variables such as the specific growth rate of fermenting organism, ethanol yield and specific ethanol production rate.

For the ethanol fermentation process considered in this work, a major limitation is apparent i.e. ethanol inhibition is appreciable. A viable approach to

countering this is by adopting simultaneous fermentation and product separation. This means that the ethanol is removed from the fermenter as it is being produced. Continuous product removal solves the problem of ethanol inhibition by keeping the concentration of ethanol below inhibitory levels so that the fermentation process can operate continuously without the growth of cells becoming severely inhibited. When this is done, it is possible to achieve a higher conversion of a more concentrated glucose feed.

NOMENCLATURE

K_s	Half saturation constant (g/l)
P	Ethanol concentration (g/l)
P_m	Maximum ethanol concentration (g/l)
S	Substrate (sugar) concentration (g/l)
X	Biomass (cell) concentration (g/l)
μ	Specific growth rate (1/h)
μ_{max}	Maximum Specific growth rate (1/h)

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