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Modelling Continuous Ethanol Fermentation: Effect of Dilution Rate and Optimisation of Substrate Consumption

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Abstract

In this study, continuous ethanol fermentation coupled with in situ gas stripping was analysed using a fermentation model. The growth of cells was simulated using the Hinshelwood specific growth rate model while the production of ethanol was simulated using the Luedeking-Piret production model. The model was simulated to investigate the effect of dilution rate on the fermentation process and substrate consumption was then optimised. Results obtained showed that the fermentation process was favoured by low dilution rates as evident in the increase in the amount of ethanol produced, growth of microbial cells and substrate utilisation. Optimisation of the continuous fermentation process resulted in a substrate utilisation rate of 97% when a dilution rate of 0.01 1/h and a stripping gas flow rate of 68.34 L/hwas used.

Keywords: Modelling, Bioethanol, Fermentation, Luedeking-Piret model, *Geobacillusthermoglucosidasius*, Gas stripping.

Nomenclature

a	interfacial area per unit volume (m^2/m^3)
C_E	Ethanol concentration (g/L)
$C_{E,M}$	Maximum ethanol concentration (g/L)
C_S	Sugar substrate concentration (g/L)
C^o_S	Inlet sugar substrate concentration (g/L)
C_X	Microbial cell concentration (g/L)
D	Dilution rate (1/h)
D_t	Diameter of fermenter (m)
F_G	Stripping gas flow rate (L/h)
F_L	Flow rate of inlet liquid stream (L/h)
H_E	Dimensionless Henry's law constant
h_{conv}	Convectional heat transfer coefficient ($W/^oC$)
k_d	Endogenous decay constant (1/h)
K_G	Mass transfer coefficient (m^2/h)
K_s	Substrate affinity constant (g/L)
m_s	Maintenance factor (g/gh)
t	time (h)
V_L	Volume of the liquid phase (L)
q_E	Specific substrate consumption rate (g/gh)
q_s	Specific ethanol production rate (g/gh)
$Y_{E/X}$	Ethanol yield (g/g)
β	Stripping factor (1/h)
μ	Specific growth rate (1/h)

1.0 Introduction

The use of ethanol fuel has gained worldwide recognition as a suitable alternative to conventional transportation fuels. Production of ethanol has traditionally been based on batch fermentations with yeast (*Saccharomyces cerevisiae*) using sucrose or starch-derived glucose as carbon substrates [1].However, batch fermentation processes are typically characterised by certain deficiencies.

The first has to do with the low productivity of the process. Achieving a high productivity in bioreactors is important in obtaining better process economics of products such as bioethanol, amino acids and single-cell proteins [2]. A high productivity level with maximal substrate utilisation and product concentration can be achieved by utilising a high cell density culture in a continuous process [3]. In practice, for this to be achievable, the dilution rate must be kept lower than the growth rate of the cells in order to avoid cell wash out.

Secondly, the production of ethanol by fermentation in a simple batch reactor is often limited by the performance of the

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fermenting microorganisms. It is an inhibitory process because the ethanol produced is toxic to the microorganisms. Typically, yeast cannot tolerate ethanol concentrations in excess of 10% w/v [4]. Because of this limitation, it is necessary to use large and often expensive fermenters. The large volume of water associated with this process leads to high cost of downstream separation and purification of ethanol. This limitation can be overcome in a number of ways. One approach is to start the process with a relatively dilute glucose solution, usually not more than 16% by weight in order to have complete conversion of the glucose substrate in a reasonable time. Another approach is to combine fermentation with product separation in a continuous process [5]. This means that the ethanol is removed from the fermenter as it is being produced. When this is done, it is possible to achieve a higher conversion of a more concentrated glucose feed. Since less water is associated with this process, the separation costs are greatly reduced. Park and Geng [6] have recently reviewed methods for combining fermentation with product separation in continuous mode. These include fermentation under vacuum [7], fermentation with product recovery via pervaporation [8], fermentation with product recovery via liquid-liquid extraction [9], fermentation with product recovery via perstraction [10] and fermentation with product recovery via adsorption [11].*In situ* gas stripping using an inert gas has been established as a viable mean of recovering ethanol from fermentation broths [4,12,13]. By adopting gas stripping as a recovery tool, the problem of product inhibition can be significantly alleviated. Furthermore, significant increase in ethanol productivity and substrate utilisation as well as reductions in the cost of product concentration and purification can be achieved [14].

Mathematical models have been used to predict the influence of operating variables on the performance of fermentation processes [15].These models could be used to develop better strategies for the optimisation of the fermentation process to ensure its economic viability. Hence, the objective of this study is to model the performance of a continuous ethanol fermentation process coupled with gas stripping and to simulate the process to determine the effect of dilution rate as well as optimising substrate consumption during fermentation.

2.0 Materials and Methods

2.1 Microorganism and Fermentation

Geobacillusthermoglucosidasius obtained from the biotechnology division of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria was used as the fermenting microorganism. The procedure adopted for the fermentation process is the same as that reported by Cripps et al. [16]. The fermentation process was carried out for 21 hours in 2 L bioreactor with a working volume of 1.5 L. Gas stripping was carried out using oxygen-free nitrogen gas.

2.2 Analytical Methods

The concentration of ethanol and fermentable sugars was determined using a gas chromatography system equipped with a flame ionisation detector (GCD-Gas chromatograph, Pye Unicam UK).

2.3 Model Formulation

The governing equations for a continuous ethanol fermentation process coupled with gas stripping in a continuous bioreactor are [17]:

Microbial cell balance:
$$\frac{dC_X}{dt} = (\mu - k_d - D)C_X \quad (1)$$

Product balance:
$$\frac{dC_E^L}{dt} = q_E C_X - (D + \beta)C_E^L \quad (2)$$

Substrate balance:
$$\frac{dC_S}{dt} = q_S C_X - D(C_S^o - C_S) \quad (3)$$

The stripping factor is defined as:

$$\beta = \frac{F_G H_E K_G a}{F_G + V_L K_G a} \quad (4)$$

D (= F_L/V_L) is the dilution rate, $\mu$is the specific growth rate, q_E is the specific production rate, and q_s is the specific substrate consumption rate. These equations are based on the following assumptions.

- The stripping gas is oxygen free
- The equilibrium distribution coefficient, H_E, and other quantities, F_G, F_L, $V_L$etc., are constant during fermentation.

The specific productivity rate of ethanol (*g/gh*) is given by a modified form of the Luedeking-Piret model [18].

$$q_E = Y_{E/X}\mu \quad (5)$$

$Y_{E/X}$ (g/g) is the ethanol yield. The specific substrate consumption rate is given by the maintenance energy model of Pirt [19].

$$q_s = \frac{\mu}{Y_{X/S}} + m_s \quad (6)$$

where $Y_{X/S}$ (g/g) and m_s (g/gh) are the yield of microbial cells and the maintenance factor respectively. The growth of cells was simulated using the growth kinetic model proposed by Hinshelwood [20] as shown in Equation (7).

$$\mu = \mu_{\max} \frac{C_S}{K_s + C_S}\left(1 - \frac{C_E^L}{C_{Em}}\right) \quad (7)$$

C_{Em} is the ethanol concentration above which the growth of microbial cells is completely inhibited.

2.4 Optimisation of Substrate Consumption

The consumption of substrate during continuous fermentation was optimised by minimising the difference between the residual sugar concentration and the initial sugar concentration in the fermenter. The objective of the optimisation exercise was to ensure that the residual sugar concentration in the fermenter was maintained at a minimum value as much as possible within the bounds of the constraints presented by the fermentation conditions. These conditions include the flow rate of the stripping gas and the dilution rate. Hence the optimisation problem was posed as a minimisation problem as follows.

Minimize (objective function): $z = (C_S - C_S^o)^2$

Subject to (constraints): $30 \le F_G \le 100$

$0.01 \le D \le 0.5$

$C_S \le 5$

$D \ge 0$

$F_G \ge 0$

The objective function (z) represents the difference between the residual sugar and the initial sugar concentration in the fermenter and this was minimised subject to the constraints presented. The first two constraints are referred to as interior point constraints because they are valid at the middle of the time horizon. The third constraint is referred to as an end point constraint because it must be satisfied at the end of the operation. The first constraint specifies that the stripping gas flow rate must be bounded between 30 and 100 with both bounds inclusive. The second constraint specifies that the dilution rate must be bounded between 0.01 and 0.5 with both bounds inclusive. The third constraint specifies that the final sugar concentration in the fermenter must not be greater than 5 g/L. The last two constraints are referred to as non-negativity constraints which mean that the two variables (gas flow rate and dilution rate) must possess non negative values. The optimisation problem was implemented in as a nonlinear programming problem (NLP) which was solved numerically using the method of Lagrange multipliers [21].

In order to investigate the dynamic behaviour of the fermentation process, the system of equations was solved numerically and the data presented in Table 1 was used for simulation.

Table 1: Parameter values for dynamic simulation

Parameter	**Value**	**Source**
Affinity constant K_s	5.423 g/L	Cripps et al. [16]
Fermenter volume V_L	1.5 L	Hild [22]
Gas phase mass transfer coefficient K_q	0.73120 m/h	Hild [22]
Henry's law constant for ethanol H_E	0.0039 L/L_{qas}	Hild [22]
Endogenous decay constant k_d	0.106 *1/h*	Amenaghawon et al. [23]
Cell maintenance factor m_s	0.376 *g/gh*	Amenaghawon et al. [23]
Ethanol yield $Y_{E/X}$	3.741 *g/g*	Amenaghawon et al. [23]
Microbial cell yield $Y_{X/S}$	0.202 *g/g*	Amenaghawon et al. [23]
Maximum specific growth rate μ_{max}	0.360 *1/h*	Amenaghawon et al. [23]
Maximum ethanol concentration C_{Em}	30.130 *g/L*	Amenaghawon et al. [23]

3.0 Results and Discussion

Figure 1 shows the effect of fermentation time and dilution rate on the amount of ethanol produced. The results show that low ethanol concentrations were obtained at high dilution rates. This observation could be attributed to the fact that at high dilution rates, the fermentation medium had a shorter residence time and since the microbial cell population would then have very little contact time with the medium, the biomass concentration will be reduced by wash out as shown in Figure 2[24].

Another consequence of high dilution rates is that with the little residence time available to the microbial cells, less of the sugar substrate will be consumed and less products will be produced during the fermentation process [25]. Similar results have been reported by other researchers [26].

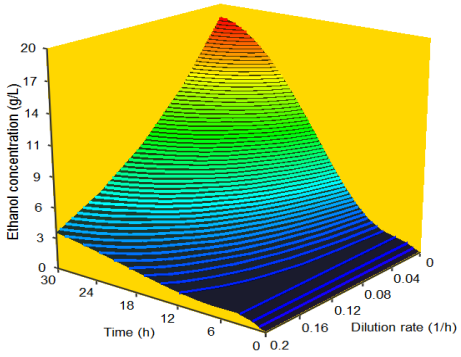

Figure 1: Effect of fermentation time and dilution rate on ethanol concentration

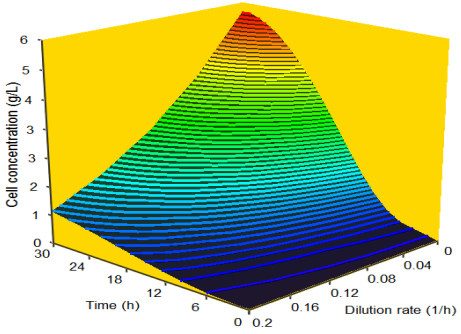

Figure 2: Effect of fermentation time and dilution rate on cell concentration

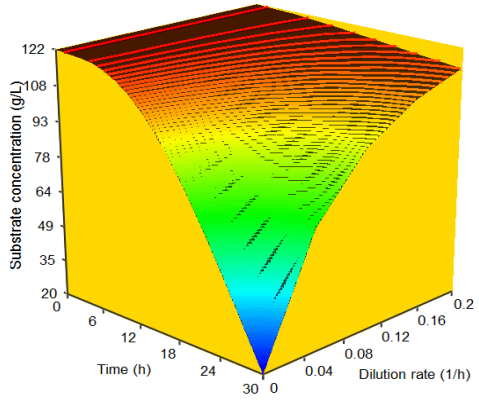

Figure 3: Effect of fermentation time and dilution rate on substrate concentration

Increasing the dilution rate resulted in a decrease in sugar substrate utilisation as shown in Figure 3. This is evident in the increase in residual substrate concentration as the dilution rate was increased. At a very low dilution rate, the substrate utilisation was estimated to be about 93%. This value however decreased to about 24% when the dilution rate was increased to 0.1 h^{-1}. A similar trend was observed by Chen et al. [25] for the production of bioethanol using immobilized *Saccharomyces cerevisiae* in a fibrous bed bioreactor. They also reported that the steady state ethanol concentration decreased with increase in the dilution rate. The dilution rate is a variable that must be given important consideration during continuous fermentation. Zanette et al. [27] reported that at low dilution rates, the fermentation medium is presented with sufficient time for the bioconversion of the sugars into ethanol. Nevertheless, the hydraulic retention time is very high and there is the possibility of the production of significant amounts of by-products. Hence there has to be a trade-off between ethanol productivity and sugar substrate utilisation to ensure continuous process operation [28].

Table 2 shows the lower and upper bounds of the constrained variables as well as the final optimal estimates of these variables. The results showed that the constraints were satisfied within the bounds specified.

Table 2: Optimal estimates of constrained variables

Constrained variables	Values		
	Optimal estimate	Lower bound	Upper bound
G (L/h)	68.34	30	100
D (1/h)	0.01	0.01	0.5
C_S *(g/L)*	3.52	0	5

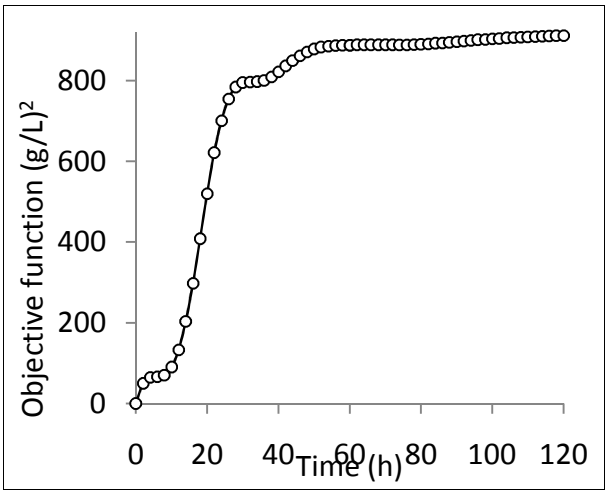

Figure 4: Response of the substrate concentration to the optimisation process

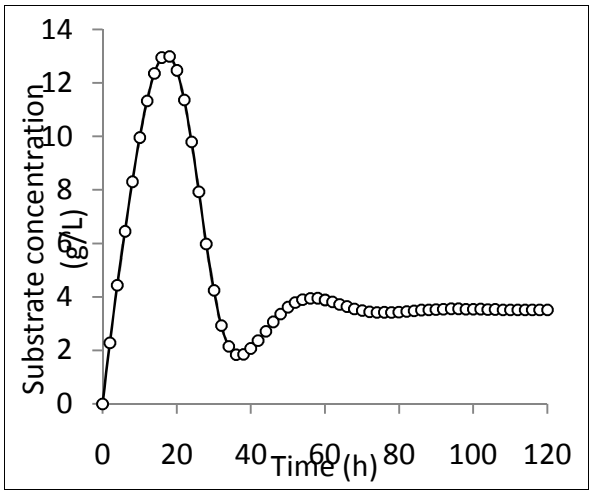

Figure 5: Response of the substrate concentration to the optimisation process

Figure 4 shows the response of the objective function in the course of the optimisation process. The objective function displayed a stable value towards the end of the optimisation run indicating the optimum point had been reached. Figure 5 shows the response of the sugar substrate concentration in the course of the optimisation process. In the course of determining the optimum values of the constrained variables (gas flow rate and dilution rate), the sugar concentration in the vessel displayed an initial increase with time to reach a maximum value of about 13 g/L after which it decreased and finally attained at steady value of about 3.52 g/L. Considering the initial sugar concentration of about 122 g/L, the final sugar concentration of 3.52 g/L represents about 97% substrate utilisation.

4.0 Conclusion

A continuous ethanol fermentation process coupled with in *situ gas* stripping was analysed in this study using a mathematical model. The model was simulated to investigate the dynamic behaviour of the process and the substrate utilisation was then optimised. To obtain high ethanol concentration, significantly viablemicrobial cell population and appreciable substrate utilisation, low dilution rates should be used.Optimisation of the continuous fermentation process resulted in a substrate utilisation rate of 97% when a dilution rate of 0.01 h^{-1} and a stripping gas flow rate of 68.34 L/h was used.The high substrate utilisation recorded as well as the optimal stripping conditions determined could serve as useful information in developing better strategies for fermentation processes to ensure its economic viability.

5.0 References

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