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Inhibitory Effect of Product and Substrate under Dihydroxyacetone Production from Glycerol

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Abstract : In this work the growth of *Gluconobacteroxidans* was evaluated in dihydroxyacetone production by fermentation of glycerol considering different substrate concentrations to identify the inhibition effect. The cell growth was measured at 20, 50 and 100 g/l of initial glycerol concentration. The rate of biomass growth was inhibited at higher glycerol, DHA and biomass concentrations. This was confirmed by the goodness of fit of a mathematical model with the experimental data that describes the entire curves in the three fermentations. The cell growth rate was inverse correlated with the biomass concentration.
Keywords: glycerol fermentation, inhibitory effect, cell growth, dihydroxyacetone, *Gluconobacteroxydans*.

1. Introduction

Dihydroxyacetone (DHA) is a ketone and carbohydrate with alternate name, glycerone.DHA is used in skin care products as selftanning agent[1,2] and active pharmaceutical ingredient for recalcitrant vitiligo[3]. Moreover, DHA can be used as precursor for other fine chemicals and pharmaceuticals agents[4].

DHA is produced, industrially,from glycerol by fermentation with acetic acid bacteria (*Gluconobacter, Acetobacter*). Other alternatives have been studied as DHA production from glucose using Escherichia coli (E. coli)[5,6] and heterogeneous catalyst with molecular oxygen as oxidant agent[7-10].

The utilization of glycerol for the synthesis of value-added chemicals[11-13] has received increasing attention due to the intensification in the production of glycerol as by-product in the biodiesel industry.

Commercial production of dihydroxyacetone is via incomplete oxidation in the fermentation of glycerol by *Gluconobacteroxydans*. This microbial process offers high selectivity with low productivity and high costs of separation and processing of DHA due to the limitations related to the inhibition effect of substrate and product[14,15].

During the glycerol fermentation by *Gluconobacteroxydans*, the demand for energy and the oxygen uptake rate (qO_2) increases proportionally with the biomass growth, driven to an acceleration of glycerol oxidation and product formation[13], that has an inhibitory effect on bacterial growth. Also, high glycerol concentration is inhibitory for DHA production only during the growth phase.

The kinetic models as Haldane, Monod[17-20], Aiba and Andrews have been used to understand the behavior of microbial growth considering the substrate concentration and inhibitory effect according with substrate [21-23].

Aim of the present study was to analyze the biomass growth at different initial concentrations of glycerol to establish the relations with biomass growth according with models of Haldane, Monod, Aiba, Andrews and postulated, that is similar to the Haldane´s model but it provides some assumptions with the biokinetic parameters.

2. Experimental

2.1 Materials and method

The materials, fermentations, growth and maintenance of G. oxydans culture were realized according to the procedure previously described[24]. During the fermentations, the concentrations of glycerol and DHA were recordedas described[25].

2.2Analytical methods

The cell culture growth was determined using a UV -VIS Scanning Spectrophotometer equipment, Model Spectro UV -2650 Brand LABOMED INC., by turbidimetric technique at 578 nm[26]. A calibration curve that related the cell concentration with absorbance was prepared[27].

2.3Growth Kinetics

Bacterial growth is described by Eqs. 1 and 2.

$$\frac{dX}{dt} = \mu X \qquad (1)$$

Where X is the concentration of biomass (g/L), μ is the specificgrowth rate (h^{-1}) and t is time (h). The Haldane equation, Eq. 2, has been proposed as a modification of Monod model for inhibition by substrate noncompetitive.

$$\mu = \frac{\mu_m S}{Ks + S + S^2/K_I} \qquad (2)$$

Whereμ_m is the maximum specific growth rate of biomass (h^{-1}), S is substrate concentration (g/L), K_S is substrate saturation constant (g/L) and K_I is the substrate inhibition constant (g/L).

Kinetic models were tested with specific growth rate. These models are shown as follows:

1. Haldanemodel
$$\mu = \frac{\mu_m S}{Ks + S + S^2/K_I}$$
2. Monod model
$$\mu = \frac{\mu_m S}{K_s + S}$$
3. Aibamodel
$$\mu = \frac{\mu_m S}{K_s + S} exp\left(-S/K_I\right)$$
4. Andrews model
$$\mu = \frac{\mu_m S}{(Ks + S)\left(1 + S/K_I\right)}$$

5. Postulated model

$$\mu = \frac{\mu_m S}{1 + K_i S + {S^2}/{K_I}}$$

Where,Ki is a factor of proportionality non-dimensional (unitless).

Knowledge of the growth kinetic of a cell culture permits to predict the course of the fermentation, the evaluation of rate of growth and yields. Also it provides useful information to establish production strategies and process optimization. The study of growth kinetic of glycerol fermentation was performed to understand the process of DHA production, operating conditions were considered as medium composition, temperature and pH, among others.

A modified equation was used to determine the biokinetic parameters of growth kinetic for each curve of substrate concentration (20, 50 and 100 g/L) with kinetic model of concentration of biomass (X) for experiments:

$$\frac{dX}{dt} = \frac{a + bC_S}{[(1 + cC]_S + dC_S^2) * X} \qquad (3)$$

Where Cs is the substrate concentration (g/L), X is the concentration of biomass (g/L) and the inhibition effect parameters are a, b, c and d.

3. Results and discussion

3.1 Cell growth kinetics

The Fig. 5 shows the increase of biomass growth concentration for fermentations of 20, 50 and 100 g/L of initial glycerol concentration. The cell growth was inhibited at experiment with 100 g/L of glycerol between the ranges of time 20 to 72 h, due to high substrate concentration, as it is observed when comparing with data of fermentation of 50 g/L in the Fig. 1, which were the higher. The biomass concentration showed an exponential growth during the first 20 hours and after a stationary phase was observed, the death phase wasn't detected, see Fig. 1. Only the lag phase was observed in fermentation of 50 g/L of glycerol. The lag phase is shorter with 20 and 100 g/L due to the inoculation.

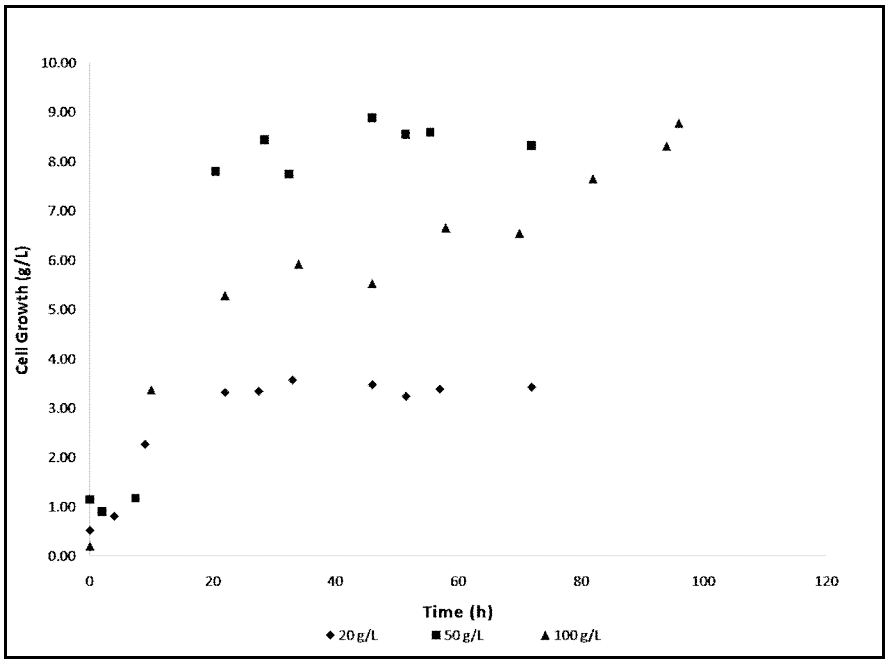

Fig. 1. Cell Growth of *Gluconobacteroxydans* at fermentation of 20, 50 and 100 g/L

3.2 Modeling the kinetics of the culture growth

The models of Haldane, Monod, Aiba, Andrews and postulated were corroborated with experimental data for fermentation at 20, 50 and 100 g/L.

The estimated biokineticparameters of K_S, K_I and μ_m are listed in the Table 1. The high correlation coefficients were obtained for the five models. However, the inhibitory effect was not considered, hence the Monod's model shown the lowest value. The Andrew's model with the postulated had the highest correlation coefficients. The Aiba's and Andrew's models and postulated achieved the best correlation coefficients. The parameter's values for K_S are in the range of 1- 115 g/L. Decreasing the values for K_S let to increasing in the values for K_I in the Aiba's models and postulated. Although, values of K_S and K_I exhibited an inverse relation in the Haldane's model, hence these values were similar in the Andrew's model.

Table 1.Estimated parameters of dihydroxiacetone at the exponential growth phase.

Model	Equation	Parameters	R^2
Haldane	$\mu = \frac{\mu_m S}{Ks + S + S^2/K_I}$	μ_m = 1.5727 K_S = 101.9994 K_I = 6.5939	0.9513
Monod	$\mu = \frac{\mu_m S}{K_s + S}$	μ_m = 0.1501 K_S = 3.5754	0.8662
Aiba	$\mu = \frac{\mu_m S}{K_s + S} exp\left(-S/K_I\right)$	μ_m = 0.3106 K_S = 14.2123 K_I = 114.4625	0.9838
Andrews	$\mu = \frac{\mu_m S}{(Ks + S)\left(1 + S/K_I\right)}$	μ_m = 0.6467 K_S = 30.6756 K_I = 30.6729	0.9903
Postulated	$\mu = \frac{\mu_m S}{1 + K_i S + S^2/K_I}$	μ_m = 0.0188 K_S = 1 K_i = 0.0479 K_I = 916.2422	0.9923

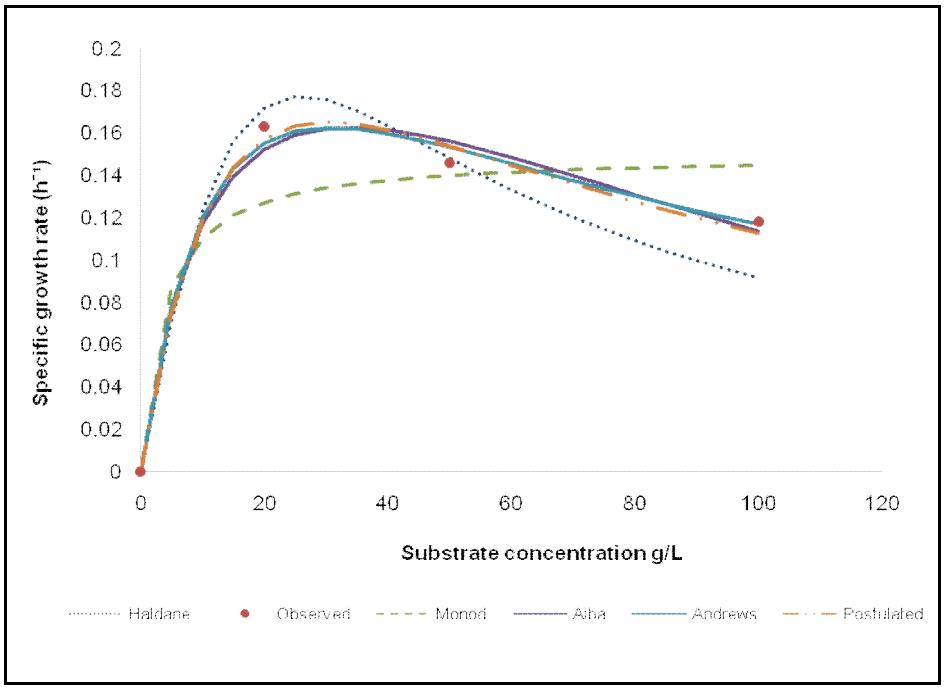

Fig. 2.Experimental data and growth model predictions

3.3 Kinetic Model

The parameters of regression nonlinear of growth curves are shown in the Table 1. According with the results, the rational regression presentshigh correlation coefficients with the experimental data for three fermentations. The high correlation coefficient of rational regression for experiment with 50 g/L provides a satisfactory description for the data, indicated by a correlation of 0.9928, as is shown in Table 2. The experimental data and those predicted by the proposed model for biomass concentration with time are presented in Fig. 3, where the biomass concentration (X) is represented as function of the time.

Table 2. Nonlinear regression for fermentations of glycerol

Initial Concentration Glycerol (g/L)	Nonlinear Regression	Parameters	R^2
20 g glycerol/L	$X = \frac{(Kg + Kn * t)}{(1 + Km * t + Ki * t^2)}$	Kg = 0.3457 Kn = 0.2541 Km = 0.0268 Ki = 0.0006	0.9675
50 g glycerol/L	$\mathbf{X = \frac{(Kg + Kn * t + Kv * t^2)}{(1 + Km * t + Ki * t^2)}}$	Kg = 1.3219 Kn = -0.4910 Km = -0.0982 Ki = 0.0071 Kv = 0.0566	0.9928
100 g glycerol/L	$\mathbf{X = \frac{(Kg + Kn * t + Kv * t^2)}{(1 + Km * t + Ki * t^2 + Kw * t^3)}}$	Kg = 0.1858 Kn = 0.8590 Kv = -0.0123 Km = 0.1325 Ki = -0.0027 Kw = $8.2811*10^{-6}$	0.9856

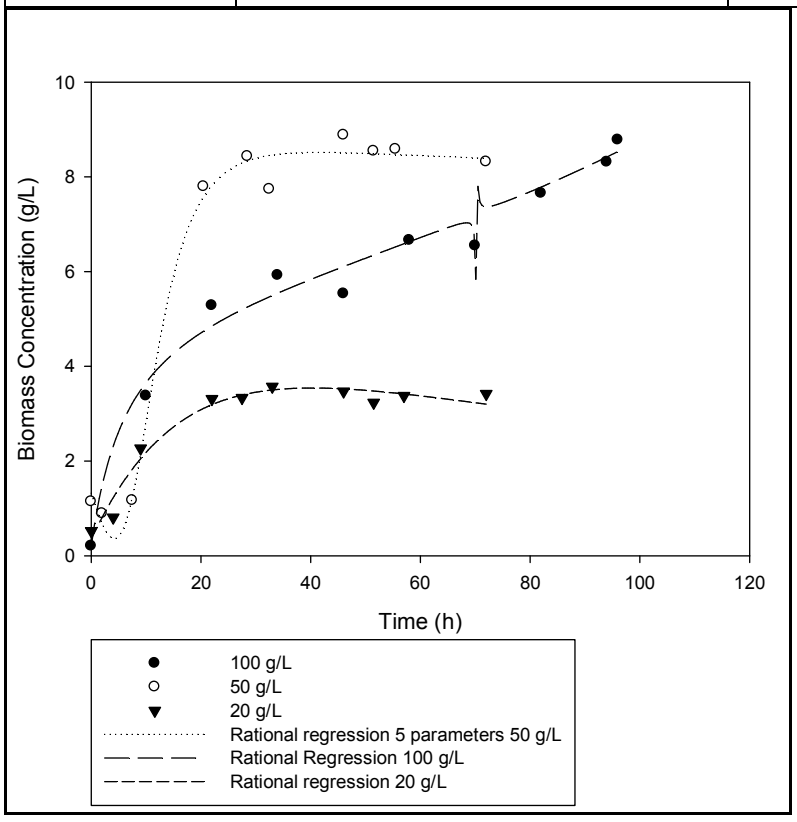

Fig. 3. Experimental data and regression nonlinear for biomass concentration as function of reaction propagation time.

Model equation for description the growth of microorganism was proposed, see equation 1, the parameters are shown in the Table 2; a goodness fit was observed with experimental data. The convenience of this equation is the representing the entire growth curve including the lag phase (for experiments with 50 g glycerol/L), the exponential growthand the stationery phase, can be described in the Eq. (3).

$$\frac{dX}{dt} = \frac{a + bC_S}{[(1 + cC]_S + dC_S^2) * X}$$

The values of rate of cell growth($\frac{dX}{dt}$) were obtained of models of nonlinear regression, which are shown in the Table 1. The concentrations of substrate (Cs) have been reported previously and are discussed in more detail elsewhere[15]. The concentration of biomass (X) is shown in Fig. 1 and 3. The proposed model exhibits an inverse relation of rate of cell growth with the biomass concentration due to inhibition.

Table 3.Non lineal regression analysis of kinetic parameters to cell growth $\left(\frac{dX}{dt}\right)$for glycerol concentration of 20, 50 and 100 g/L.

Experiment	Kinetic Parameters	R^2	95% confidence
100 g/l rational regression	a= 474.7 b= 3.7953 c= -33.9276 d= 0.5792	0.9768	0.0336 5.522e-04 1.2E-05 2.089e-07
50 g/l rational regression	a= 0.2199 b=-0.0050 c= -0.0458 d= 5.468e-04	0.9545	1.779e-06 3.905e-08 1.11e-07 2.678e-09
20 g/l rational regression	a= -0.1299 b= 0.2459 c= 0.0566 d= 0.0891	0.9466	2.612e-05 1.325e-05 7.334e-05 6.185e-06

4. Conclusions

The biomass concentration showed an exponential growth during the first 20 hours, at three fermentations (20, 50 and 100 g/L of initial glycerol) and after a stationary phase was observed. Similar observation was reported for production of DHA[25].

The Haldane, Monod, Aiba, Andrews and postulated model's predicted the experimental data. However, the Monod model presented the lower correlation coefficient due the absence of inhibitory parameter.

The values of the substrate saturation constant (K_S)are in the range of 1- 115 g/L. The maximum specific growth rate of biomass, $\mu_m$was found in 0.0188, 0.1501, 0.3106, 0.6467 and 1.5727 h^{-1} for the models of postulated, Monod, Andrews and Haldane, respectively.

A model equation was proposed for description of cell growth showing goodness fit with experimental data and describing the entire curves in the three fermentations. The biokinetic parameters were shown low values of confidence, lower to 0.05, which support the function of evaluated model. The cell growth rate was related to biomass concentration inversely.

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