

Kinetics of gluconic acid production and cell growth in a batch bioreactor by *Aspergillus niger* using breadfruit hydrolysate

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Abstract

The kinetics of gluconic acid production by *Aspergillus niger* (*A. niger*) using breadfruit hydrolysate was studied in a batch bioreactor. A simple model of Luedeking–Piret, logistic, and Luedeking–Piret-like equations were proposed for gluconic acid production, cell growth, and breadfruit hydrolysate consumption respectively. The maximum gluconic acid concentration (Pt) of 109.95 g/L with 0.967 g/g process yield (88.70%) was obtained for the medium containing 120 g/l breadfruit hydrolysate at 2 vvm aeration rate. For the same conditions, the biomass concentration and maximum specific growth were obtained as 24.3 g/L and 0.018 hr⁻¹ respectively. The models proposed for the gluconic acid production was sufficiently satisfactory, since the theoretical values of Pt (100.94 g/L) and biomass concentration (23.43 g/L) obtained from the models were almost the same with the experimental values. The model for the breadfruit hydrolysate utilization gave R² and Adj. R² of above 0.840 and 0.660 respectively; an indication that the model is suitable for the production of gluconic acid. Therefore, the concentration of initial breadfruit hydrolysate as substrate and aeration rate have shown significant effect on gluconic acid production and *A. niger* accumulation. The evaluation of the models showed that the gluconic acid production was growth-associated.

Practical applications

Breadfruits, a perishable fruit has been abundantly found in Ile-Ife, Nigeria where 50% of it perishes due to its short shelf-life. This study therefore provides alternative use for breadfruit, in order to reduce its wastage. Furthermore, the growing demand of gluconic acid can be met through the use of this cheap and available breadfruit. The findings in this study can be employed to develop a bioreactor for fermentation of breadfruit hydrolysate to produce gluconic acid.

KEYWORDS

Aspergillus niger, breadfruit, fermentation, gluconic acid, hydrolysis

1 | INTRODUCTION

Gluconic acid, also known as pentahydroxycaproic acid, is a non-corrosive, non-volatile, non-toxic, mild acid produced from glucose through a simple dehydrogenation reaction catalyzed by glucose oxidase (Ganguly, Patra, & Mandal, 2010; Ramachandran, Fontanille, Pandey, & Larroche, 2006). The simple dehydrogenation reaction takes place without involvement of complex metabolic cell pathways, which is realized with a high selectivity, high rate, and high yield of conversion (Znad, Markos, & Bales, 2004a). Gluconic acid and its salts are important materials widely used in pharmaceutical, food, animal feed, detergent, textile, leather, photographic, and con-

crete industries (Betiku, Ajala, & Layokun, 2011; Ramachandran et al., 2006).

The gluconic acid can be produced using different methods such as: chemical, electrochemical, biochemical, and bio-electrochemical (Betiku et al., 2011; Liu, Weng, Zhang, Xu, & Ji, 2003). The biochemical method through fermentation has many advantages for the gluconic acid production, which includes: low production cost, mild reaction conditions, and the use of renewable resources as raw materials. Other advantage is the conversion of nearly 100% of glucose to gluconic acid under appropriate conditions (Anastassiadis, Kamzolova, Morgunov, & Rehm, 2008). These make the process exclusively useful for commercial production of gluconic acid, due to its high and still growing demand of about 60,000

tons per annum in United States (Betiku et al., 2011; Shindia, El-Sherbeny, El-Esawy and Sheriff, 2006; Singh, Jain, & Singh, 2003). Several microbial species such as *A. niger* and *Gluconobacter oxydans* have been utilized in many researches of gluconic acid production through fermentation (Akbarningrum, Rudy, & Lindawati, 2009).

Successful efforts have been made in using some agro-food by-products (grape must, banana must, and sugarcane molasses) for production of gluconic acid (Ikeda, Park, & Okuda, 2006; Rao & Panda, 1994; Vassilev, Vassileva, & Spassova, 1993). Gluconic acid production by *A. niger* is an aerobic fermentation with high oxygen demand (Znad et al., 2004a) and sensitive to other factors such as pH and temperature. This requires mathematical modeling to describe the process in a simple manner with adequate representation of the process.

Mathematical modeling of fermentation process gives understanding of the relationship among principal state variables; provides quantitative information on the behavior of the process. In modeling fermentation systems, it is relatively easy to set up a system of differential equations to represent the bioprocess dynamics. The most difficult part in modeling the fermentation process is obtaining the form of the specific growth rate expression (μ) that best represents the observed kinetic behavior for the produced gluconic acid (Znad, Blazej, Bales, & Markos, 2004b). Fermentation models for a microbial process are normally divided into two classes: structured models where intracellular metabolic pathways are considered and unstructured models where the biomass is described by one variable (Liu et al., 2003).

Structured model considers cell structure, function, and composition, while unstructured model considers only cell mass to describe the biological system. Structured model seems complicated for normal use, but can be used to describe and give better understanding of the process. It is especially important when scaling up the process to achieve optimum product formation. The model can be used to predict performance under different process conditions, process optimization and process control, and in doing so, reduces development time and cost (Bouguettoucha, Balannec, & Amrane, 2011).

The development of kinetic models involve comparison of assumed models with experimental data, to obtain more relevant information and predict the behavior of process. In this study unstructured models for product formation, cell growth and glucose consumption were employed for the fermentation process of breadfruit hydrolysate to produce gluconic acid by *A. niger* in a batch bioreactor of 7 L for kinetic study.

2 | MATERIALS AND METHODS

2.1 | Materials

The matured, but not ripe breadfruits were freshly harvested from its tree in Ile-Ife, Osun State, Nigeria. The fruits, after harvesting, were washed with clean water and sliced to pieces using stainless steel knife. The sliced fruits were sun-dried until constant weight was attained and ground to fine powder. The enzymes [α -amylase (E.C.3.2.1.1) (source: *Bacillus licheniformis*) and glucoamylase (E.C.3.2.1.3) (source: *A. niger*)] were provided by Federal Institute of Industrial Research Oshodi (FIRO), Lagos, Nigeria, which were used for hydrolysis of breadfruit. The pure culture of

A. niger strain was obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The pre-culture medium was prepared at 200 rpm and 30°C using the environment-controlled incubator shaker manufactured by New Brunswick Scientific Co. (The United States). A 7 L bioreactor manufactured by New Brunswick Scientific Co. (The United States) was also used for the study.

2.2 | Methods

2.2.1 | Hydrolysis of breadfruit

Modified method of Betiku & Ajala (2010) was used for the hydrolysis of breadfruit to obtain breadfruit hydrolysate for the production of gluconic acid.

2.2.2 | Microorganism and its inoculation

The *A. niger* strain was sub-cultured on potato dextrose agar at 30°C for 7 days; the spores formed were stored in a refrigerator at 4°C with regular sub-culturing. The pre-culture medium was made up of glucose, 50 g/L; yeast extract, 3 g/L; malt extract, 3 g/L; polypeptone, 5 g/L and calcium carbonate, 20 g/L (Betiku et al., 2011). Aseptically harvested spores from the sporulating surface was taken and inoculated into 250 mL Erlenmeyer flask containing 50 mL of pre-culture medium composition. The inoculated flasks were shaken continuously using the incubator shaker at 200 rpm and 30°C for 18 hr before it was used for the fermentation process.

2.2.3 | Fermentation

The production medium for gluconic acid was 100 or 120 g/L of breadfruit hydrolysate as carbon source; Urea 1.35 g/L; Na_2HPO_4 , 0.2 g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 g/L, and calcium carbonate, 60 g/L (Betiku et al., 2011). Aerobic batch mode of fermentation was performed at 30°C for 72 hr with 12 hr sampling interval. The initial pH of the medium was adjusted to 5.5 and agitated at 300 rpm throughout the process. The study was carried out at varying aeration rates of 1 and 2 vessel volumes per minute (vvm).

2.2.4 | Analytical methods

The modified method described by Betiku et al. (2011) and Ikeda et al. (2006) was employed to determine the gluconic acid concentration. The modified 3,5-Dinitrosalicylic acid (DNS) assay method described by Betiku & Ajala (2010) was used to estimate the reducing sugar concentration. The dry weight of mycelium was obtained for biomass concentration using filter paper assay method for *A. niger* growth (Singh & Singh, 2006).

2.2.5 | Kinetic models

The Luedeking–Piret model was used to study the gluconic acid production and cell growth in a batch bioreactor. The model considered rate of gluconic acid production (P), biomass growth (X), and substrate utilization (S) to describe the fermentation process. Microsoft Excel was employed to determine the various parameters measured for the kinetic study.

2.2.5.1 | Product formation

The kinetics of product formation is the combination of both rate of growth associated and non-growth associated as was described by Pazouki, Najafpour, & Hosseini (2008). The rate of product formation is

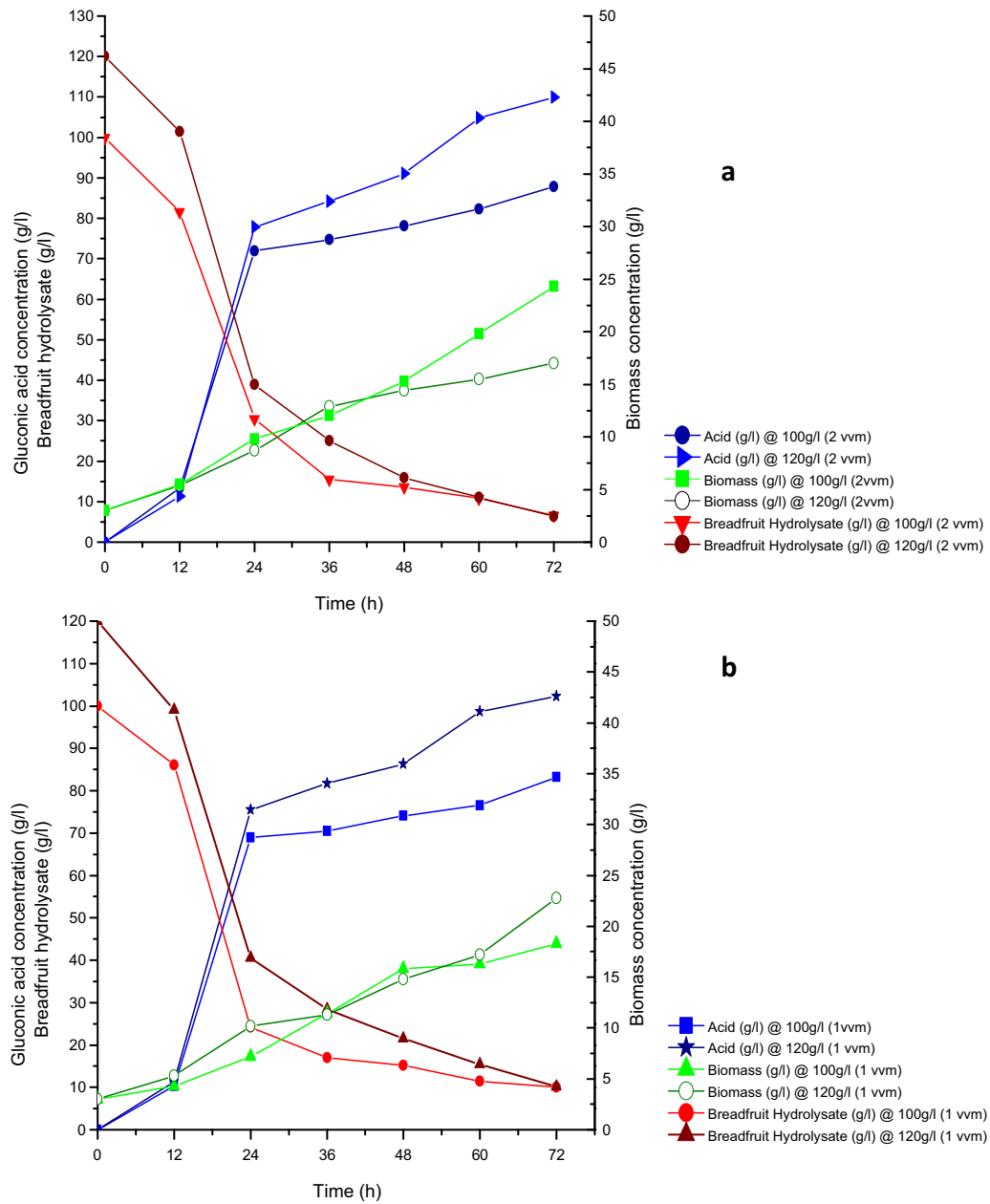


FIGURE 1 Effect of initial hydrolysate concentrations of 100 and 120 g/L at (a) aeration of 2 vvm and (b) 1 vvm on gluconic acid accumulation (g/L) and biomass concentration (g/L)

thus based on Leudeking–Piret equation shown in Equation 1a. Based on this model, the rate of product formation depends on the biomass concentration, X , and growth rate, dX/dt as in Equation 1a (Luedeking & Piret, 2000).

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (1a)$$

where α and β are the growth and non-growth associated production rate parameters respectively. For this study, gluconic acid production was considered as growth related model, therefore, $\alpha \neq 0$, $\beta = 0$. Thus, expressing Equation 1a as:

$$P = \alpha X + K \quad (1b)$$

Then, the percentage yield of gluconic acid ($\%Y_{acid}$) was obtained by using Equation 1c,

$$Y_{acid} = \frac{Y'ps}{\text{theoretical yield}} \quad (1c)$$

whereas, the theoretical yield from the stoichiometric equation was given as 1.09 gram gluconic acid per gram carbon substrate.

2.2.5.2 | Biomass growth

The logistic equation was used for describing the cell growth of the process. The equation is a substrate and product independent model, and is thus stated as Equation 2a.

$$\frac{dX}{dt} = \mu_m X \left(1 - \frac{X}{X_m} \right) \quad (2a)$$

Equation 2a was solved by integration at $X = X_0$ at $t = 0$ to obtain Equation 2b

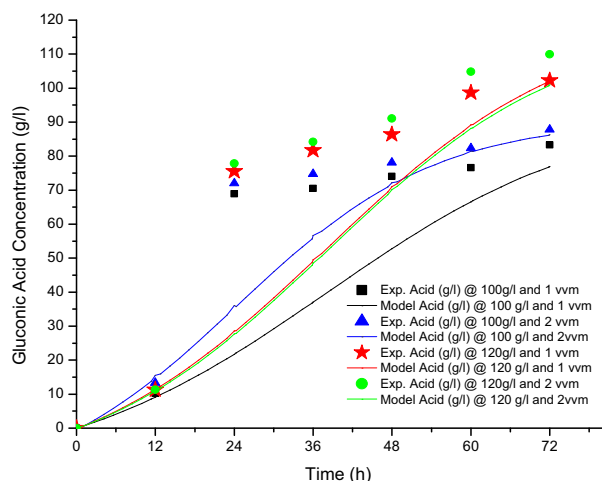


FIGURE 2 Plot of experimental data (bullet) and model data (line) for kinetic model of gluconic acid concentration against time

$$\frac{1}{X} - \frac{1}{X_m} = \frac{(X_o - X_m)\ell^{\mu_m t}}{X_m X_o} \quad (2b)$$

Equation 2b was further represented as Equation 2c

$$y = A\ell^{\mu_m t} \quad (2c)$$

where; $y = \frac{1}{X} - \frac{1}{X_m}$, $A = \frac{(X_o - X_m)}{X_m X_o}$; where X = biomass concentration, t = time, μ_m = maximum specific growth rate (hr^{-1}) of the organism and X_m = maximum biomass concentration (g/L dry weight).

2.2.5.3 | Substrate utilization

Breadfruit hydrolysate was used as a carbon source for microbial stability and maintenance, as well as for gluconic acid production. The substrate consumption equation is thus expressed by Leudeking–Piret kinetic that neglects the amount of carbon substrate used for product formation (Akbaringrum et al., 2009) which is given as Equation 3a

$$\frac{dS}{dt} = m_s X + \frac{1}{Y_{x/s}} \frac{dX}{dt} \quad (3a)$$

Substituting Equation 2b into Equation 3a and solved gave Equation 3b

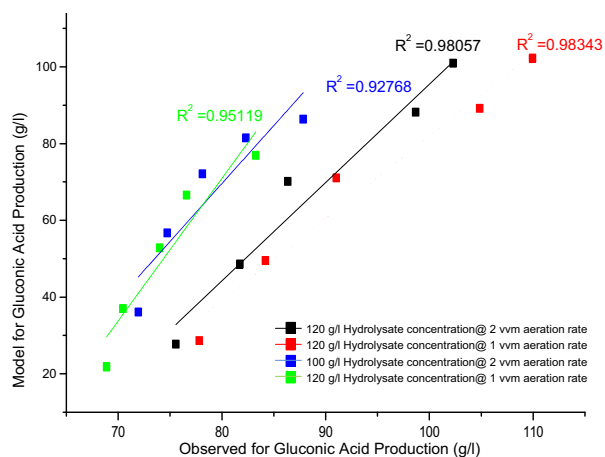


FIGURE 3 Plot of model data against observed data for gluconic acid concentration

TABLE 1 Kinetic parameters for gluconic acid production from breadfruit hydrolysate using *A. niger*

BHC	AR	Y_{ps}	%Yield	Y_{px}	Q_p	Q_s	$Y_{x/s}$	μ
100	1	0.925	84.88	4.165	1.1536	1.2738	0.1611	0.0239
100	2	0.940	85.76	5.09	1.4655	1.5345	0.1603	0.0217
120	1	0.932	85.48	5.421	1.213	1.307	0.1563	0.018
120	2	0.967	88.70	5.884	1.5778	1.6221	0.1336	0.0179

%Yield, percentage yield of gluconic acid; Y_{ps} (g/g), process product yield; Y_{px} (g/g), specific product yield; μ (hr^{-1}), specific growth rate; $Y_{x/s}$ (g/g), growth yield coefficient; Q_s (g/L/hr), volumetric rate of substrate consumption; Q_p (g/L/hr), volumetric product formation; AR, aeration rate (vvm); BHC, breadfruit hydrolysate concentration (g/L).

$$S_o - S = \frac{X - X_o}{Y_{x/s}} + X_m m_s t + \frac{X_m m_s}{\mu_m} \ln \left(\frac{X_o}{X} \right) \quad (3b)$$

From Equation 3b, the values of " $Y_{x/s}$ " and m_s were determined by plotting the graph of " $S_o - S$ " against " t ", where X_o is inoculum's biomass concentration (g/L) and S_o is initial carbon substrate concentration (g/L).

The Equations 1b, 2b, and 3b were used to simulate the experimental results for gluconic acid formation (product), biomass growth and substrate utilization respectively, to evaluate kinetic parameters for initial hydrolysate concentrations of 100 and 120 g/L at aeration rates of 1 and 2 vvm.

3 | RESULTS AND DISCUSSION

3.1 | Experimental study

3.1.1 | Effect of breadfruit hydrolysate concentration on gluconic acid and biomass accumulation

Figures 1a and 1b show the effect of 100 and 120 g/L of initial breadfruit hydrolysate concentrations at 2 and 1 vvm aeration rates on gluconic acid production and biomass accumulation. The figures show that *A. niger* was able to metabolize the breadfruit hydrolysate for gluconic acid formation and biomass growth. It consumed about 80% of the hydrolysate within 48 hr of cultivation, with sharp decline in substrate curve, while the biomass concentration was increasing gradually. By 72 hr of cultivation, the hydrolysate was almost utilized (Figures 1a and 1b). This showed that the *A. niger* consumed the hydrolysate appreciably well to produce gluconic acid. The profile for biomass concentration against time was the same for the four cases considered. Gluconic acid formation increases from the beginning of the cultivation up until the hydrolysate was almost completely exhausted for all the cases studied. This observation suggests that there was a direct correlation between the breadfruit hydrolysate consumption and the gluconic acid formation. From the figures, gluconic acid production increases throughout the period of observation. The gluconic acid concentration for 100 and 120 g/L initial hydrolysate concentrations were 83.27 and 102.29 g/L for 1 vvm; and 87.83 and 109.95 g/L for 2 vvm respectively. The Y_{acid} was 84.88% and 85.48% for 1 vvm, and 85.76% and 88.70% for 2 vvm at 100 and 120 g/L initial hydrolysate

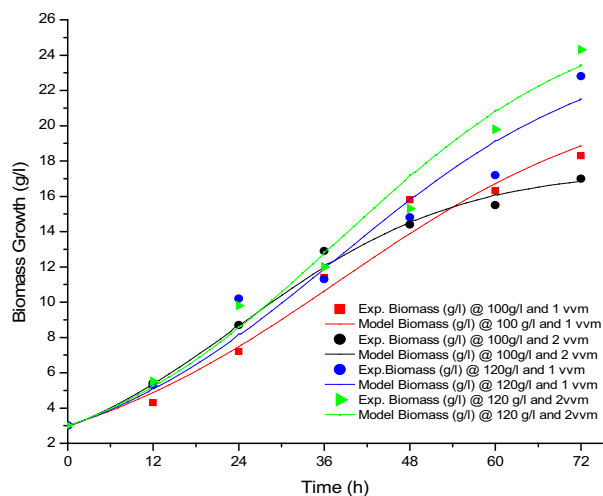


FIGURE 4 Plot of experimental data (bullet) and model data (line) for kinetic model of biomass growth against time

concentrations respectively, after 72 hr. It was observed that the initial hydrolysate concentration have significant effect on the gluconic acid production, as the increase in the hydrolysate concentration brought about increase in the percentage yield of gluconic acid. These results indicate that the high level of 120 g/L concentration of breadfruit hydrolysate enhanced the productivity of gluconic acid compared with 100 g/L breadfruit hydrolysate, which agrees with the findings in literature (Sankpal & Kulkarni, 2002). This rise in the gluconic acid concentration is attributed to stimulation of mycelial Glucose Oxidase (GOD) at increased glucose concentration (Sankpal & Kulkarni, 2002). The percentage yields obtained indicate that *A. niger* successfully metabolized the breadfruit hydrolysate to produce high yield of gluconic acid. The yields obtained were greater than those reported by Akbarningrum et al. (2009) when waste paper was used as carbon source. These results also showed that the initial hydrolysate concentration has effect on the biomass growth. It was observed that as the initial hydrolysate concentration increased, the biomass concentration also increased.

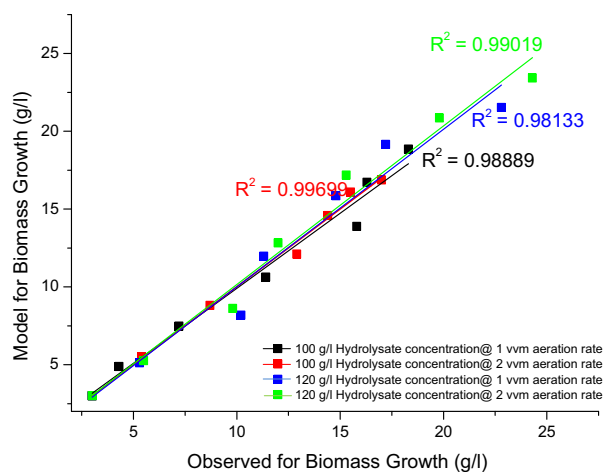


FIGURE 5 Plot of model data against observed data for biomass growth

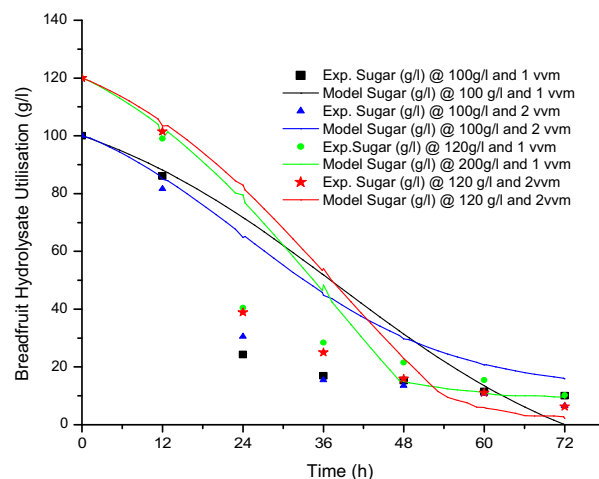


FIGURE 6 Plot of experimental data (bullet) and model data (line) for kinetic model of breadfruit hydrolysate utilization against time

3.1.2 | Effect of aeration rate on gluconic acid production and biomass growth

Figures 1a and 1b also showed the effect of 2 and 1 vvm aeration rates at 100 and 120 g/L of breadfruit hydrolysate concentrations on gluconic acid and biomass accumulation. The peak of biomass concentration that was observed ranges from 17 to 24.3 g/L for the four categories considered. From the figures, the biomass concentrations were 17.0 and 21.30 g/L at 2 vvm and 18.3 g/L and 22.8 g/L at 1 vvm, for 100 and 120 g/L of the hydrolysate concentrations respectively. These results showed that aeration rate of 1 vvm accumulate more biomass concentration than at 2 vvm. Sharma, Vivekanand, & Singh (2008) obtained similar result of 23.3 g/L biomass at an aeration rate of 0.5 vvm. Furthermore, from these results, it can be said that the production of gluconic acid was influenced by aeration. An aeration level of 2 vvm led to higher level of production with gluconic acid production of 87.83 g/L (85.76%) and 109.95 g/L (88.70%) at 100 g/L and 120 g/L respectively. These results compared favorably well with the value reported by Sharma et al. (2008), which showed that the supply

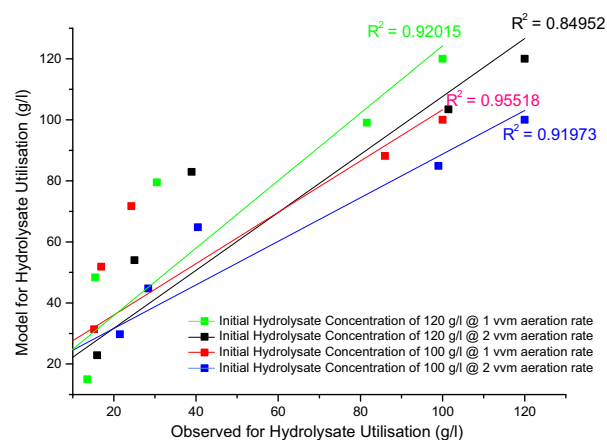


FIGURE 7 Plot of model data against observed data for breadfruit hydrolysate utilization

of oxygen at desired level is of prime significance and a major modulator for gluconic acid production (Singh & Singh, 2006).

3.2 | Kinetic study

3.2.1 | Gluconic acid production

Figure 2 shows the experimental data (bullet) and model fitted (line) for gluconic acid concentration against time (Equation 1b). This figure presents the gluconic acid production using 100 and 200 g/L of hydrolysate at 1 and 2 vvm aeration rates. Figure 3 shows the plot of model result against observed result for gluconic acid concentration. The figure revealed that the coefficients of determination (R^2) and Adjusted coefficients of determination (Adj. R^2) of the model were above 0.920 and 0.810 respectively, for the four different categories studied. These showed that the experimental data perfectly fitted the model Equation 1b. According to the fitted model for gluconic acid production, the calculated values of P_t (76.93, 86.31, 103.23, and 100.94 g/L) were quite satisfactory with the experimental value P_t (83.27, 87.83, 102.29, and 109.95 g/L) for 100 g/L at 1 vvm, 100 g/L at 2 vvm, 200 g/L at 1 vvm, and 200 g/L at 2 vvm respectively. The medium with 100 and 120 g/L of hydrolysate concentration gave process product yield (Y'_{ps}) of 0.925 and 0.932 g/g respectively, at 1 vvm; 0.940 and 0.967 g/g respectively, at 2 vvm as shown in Table 1. The volumetric gluconic acid formation obtained were 1.154 and 1.466 g/L/hr for 100 g/L (1 and 2 vvm) and 1.213 and 1.578 g/L/hr for 120 g/L (1 and 2 vvm) (Table 1).

3.2.2 | *Aspergillus niger* accumulation

Figure 4 presents the experimental data (bullet) and model fitted (line) for biomass concentration against time. Figure 5 shows the plot of model result against observed result for biomass growth of *A. niger* which gave R^2 and Adj. R^2 of above 0.981 and 0.950 respectively, for all the cases studied. These showed that the experimental data perfectly fitted the model Equation 2b. According to the fitted growth model, the calculated values of biomass concentration were quite satisfactory with the observed values as shown in the figure. The maximum specific growth rate (μ_m) obtained were 0.024, 0.022, 0.018, and 0.018 hr^{-1} for 100 g/L (1 and 2 vvm) and 120 g/L (1 and 2 vvm) respectively, as shown in Table 1. The extent to which the *A. niger* was able to utilize the breadfruit hydrolysate was estimated as the specific growth rate (μ) and coefficient of biomass yield (Y'_{xs}). The growth yield coefficient (Y'_{xs}) obtained were 0.161 and 0.160 g cell g^{-1} for 100 g/L (1 and 2 vvm respectively) and 0.156 and 0.134 g cell g^{-1} for 120 g/L (1 and 2 vvm respectively).

3.2.3 | Breadfruit hydrolysate utilization

Figure 6 shows the experimental data (bullet) and model fitted (line) of hydrolysate concentration against time with about 75% of the hydrolysate consumed within 24 hr of the experiment. Figure 7 shows the plot of model result against observed result for breadfruit hydrolysate utilization. The figure showed that the R^2 and Adj. R^2 of the model were above 0.840 and 0.660 respectively, for all the cases studied. These showed that the experimental data perfectly fitted the model Equation 3b. The volumetric rate of substrate consumption (Q_s) obtained were

1.273 and 1.535 g/L/hr for 100 g/L (1 and 2 vvm) and 1.307 and 1.622 g/L/hr for 120 g/L (1 and 2 vvm) (Table 1), which is greater than the report of Ikeda et al. (2006). This is because breadfruit hydrolysate gave better Q_s than saccharified waste paper.

4 | CONCLUSION

The breadfruit hydrolysate used in this study as the sole carbon source for the cultivation of *A. niger* gave promising results as the hydrolysate does not show any negative effect on the gluconic acid production and hence could be used as an alternative carbon source. This study has shown that *A. niger* is capable of producing gluconic acid from breadfruit hydrolysate in a batch bioreactor process. The initial hydrolysate concentration and aeration rate have shown appreciable effect on gluconic acid production and biomass growth of *A. niger*. The kinetic models sufficiently described the process, as the models results compared favorably well with the experimental results.

NOMENCLATURE

m	maintenance coefficient [g substrate (g cells h) $^{-1}$]
P	Final gluconic acid concentration (g/L)
P_0	Initial gluconic acid concentration (g/L)
S	Final substrate concentration (g/L)
S_0	Initial substrate concentration (g/L)
t	Time of fermentation (hr)
X	Cell concentration (g/L)
X_m	Maximum cell concentration (g/L)
X_0	Initial cell concentration (g/L)
$Y_{x/s}$	Yield factor for cells on carbon substrate [g cells (g substrate) $^{-1}$]
α	Growth-associated product formation coefficient (g/g)
β	Non-growth-associated product formation coefficient (g/g/hr $^{-1}$)
μ_m	Maximum specific growth rate (hr $^{-1}$)

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