Nigerian Journal of Technology (NIJOTECH)
Vol. 33. No. 2, April 2014, pp. **222 – 229**

University of Nigeria, Nsukka, ISSN: 1115-8443
www.nijotech.com
http://dx.doi.org/10.4314/njt.v33i2.12

DYNAMIC MODELLING AND SIMULATION OF CITRIC ACID PRODUCTION FROM DILUTE ACID HYDROLYSED CORN STARCH USING *ASPERGILLUS NIGER*

N. A. Amenaghawon[1,*], O.U. Osazuwa[2] and C.O. Okieimen[3]
[1, 2, 3] DEPARTMENT OF CHEMICAL ENGINEERING, UNIVERSITY OF BENIN, PMB 1154, BENIN CITY. NIGERIA
E-mail addresses: [1] *andrew.amenaghawon@uniben.edu,* [2] *osarieme.osazuwa@uniben.edu,*
[3] *cookieimen@uniben.edu*

ABSTRACT
The modelling of batch production of citric acid from corn starch hydrolysate using Aspergillus niger ATCC 9142 was carried out in this work. A validated mathematical model was developed to describe the process. Four kinetic models, Monod, Haldane, logistic and hyperbolic for simulating the growth of the Aspergillus niger cells were explored. The validity of the models in terms of predicting the growth of Aspergillus niger cells was determined by fitting each kinetic model to experimental data. Comparison of experimental results to model predicted results revealed that only the hyperbolic model was able to accurately replicate the experimental results. This was evident from the high level of correlation between the experimental and model predicted results. The kinetic parameters for cell growth, substrate consumption and product formation μ_{max}, $Y_{x/s}$, $Y_{p/x}$, K_s ***and*** K_p ***as calculated by the hyperbolic model were 0.0130h^{-1}, 0.711g/g, 13.671g/g, 0.001g/L, and 0.257 g/L respectively. Results of simulating the model showed that the production of citric acid was a growth associated process. Optimum pH, initial sugar concentration and temperature for citric acid production obtained were5.5, 40w/v and 30^{o}C respectively.***

Keywords: *Citric acid, Fermentation, Corn starch, Hydrolysate, Modelling, Aspergillus niger.*

1. INTRODUCTION
Citric acid is present in essentially all plants and in many animal tissues and fluids. It is a constituent of wine, milk, cheese and it is abundant in most citrus fruits such as oranges, tangerines, lemon, berries, lime etc. It is also a metabolic product formed in the citric acid or Krebs cycle [1, 2].

The annual production rate of citric acid stands at about 1.4 to 1.5 million tons and its demand is estimated to be growing at a rate of about 3.5 to 4.0% annually [3]. Satisfying this demand through chemical or synthetic production of citric acid has been demonstrated to be unsustainable [4, 5]. Hence, a large proportion of the world's demand for citric acid is satisfied through biotechnological means. Presently, submerged fermentation of sugar containing substrates appears to be the most economical way of producing citric acid by *Aspergillus niger* [6, 7].

In trying to study and understand the dynamic behaviour of a process, it is important to formulate dynamic models of such processes. These models upon calibration and validation will provide insights as to how the process functions, how it responds to changes in operating procedure and how amenable it is to control [8, 9].The usefulness of a dynamic mathematical model in analysing complex processes cannot be over emphasized. Dynamic modelling and simulation of processes leads to vast improvements in process economics, design, operation and control while model predictions also make it possible to identify optimal design and operational parameters and this consequently leads to the maximisation of the system's performance [10, 11].

In this work, the concept of dynamic behaviour of process systems and how a formulated model can be used to predict the behaviour of a process is presented. The case considered for investigation was the production of citric acid from acid and enzyme hydrolysed corn starch using *Aspergillus niger*. Experimental data from batch fermentation of citric acid was analysed in order to develop a model that can replicate the experimental data. The validated model was implemented in an advanced equation oriented process modelling software. Simulation of the

*** Corresponding author, Tel: +234-806 927 5563**

validated model will provide insight as to how the process responds to changes in operating procedure and how certain operating variables affect the process.

2 MATERIALS AND METHODS

2.1 Microorganism

Aspergillus niger ATCC 9142 was obtained from the biotechnology division of the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. It was maintained on Potato Dextrose Agar (PDA) slants and stored in a refrigerator at 4^{o}C until it was needed.

2.2 Substrate and Pre-treatment

Industrial grade corn starch was obtained from the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. 40% w/v corn starch was prepared by mixing the appropriate amount of corn starch with 100 mL of 0.1M hydrochloric acid. The mixture was autoclaved at 121^{o}C for a period of 15 minutes. The mixture was then removed from the autoclave and allowed to cool to ambient temperature and 1.0 M sodium hydroxide was added to stop the hydrolysis reaction. The hydrolyzed starch was filtered and the filtrate was collected for citric acid production.

2.3 Culture Medium, Inoculum and Fermentation

The constitution (g/L) of the fermentation medium used for citric acid production was as described by Lotfy et al.[12]. The pH of the culture medium was adjusted to 5.5 by adding a sterile solution of hydrochloric acid. Conidia suspensions of fungal strains were obtained from cultures grown on potato dextrose agar slants at 30^{o}C for 5 to 7 days. The spores were washed with sterilized 0.8% Tween 80 solution by shaking vigorously for 1 minute. Spores were counted with a haemocytometer to obtain approximately 1×10^{8} spores/mL. Surface fermentation was carried out in 250 mL Erlenmeyer flasks. The flask containing the fermentation medium was inoculated with 0.5mL of the inoculum and then incubated at 30^{o}C.

2.4 *Analytical Methods*

Liquid samples were taken from the fermentation vessel at intervals of 24 hours and analysed for glucose, microbial cells, citric acid and pH. Cell concentration was measured by dispensing 5 mL 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 a standard curve of dry weight of *Aspergillus niger* cells. The glucose content of the sample was determined using the method of Miller [13]. The citric acid content of the sample was determined using the method of Marier and Boulet [14]. The pH of the sample was determined using a Unican 9450model pH meter.

2.5 Batch Fermenter Model Formulation

In order to predict the citric acid productivity of *Aspergillus niger* in a batch fermenter, a mathematical model was developed. The parameters for microbial cell growth, substrate consumption and citric acid formation kinetics were estimated as part of the model validation exercise. The model was used to predict microbial cell, substrate and citric acid concentrations as well as dynamic response of the batch fermentation process. The development of the model involved deriving expressions for material, energy balances and microbial growth rates.

2.5.1 Substrate Material Balance

In carrying out the material balance, a batch fermenter was considered. This was approximated as a perfectly mixed continuous stirred tank reactor (CSTR) without inlet and outlet streams. In carry out the overall balance for the substrate, it was assumed that the volume of the fermenter was constant and that the content of the fermenter was perfectly mixed. Overall substrate balance about the fermenter is given as:

$$\frac{dS}{dt} = -q_s X \tag{1}$$

S (g/L) and X (g/L) are the concentration of substrate and microbial cells in the fermenterrespectively. The specific substrate consumption rate q_s (g substrate/g biomass/h) was given by a modified form of the maintenance model proposed by Pirt [15].

$$q_s = \frac{\mu}{Y_{x/s}} \tag{2}$$

$Y_{x/s}$(g biomass/g substrate) is the biomass yield

2.5.2 Microbial Cells Material Balance

In carrying out the overall balance for microbial cells, it was assumed that microbial growth was only limited by the availability of organic substrate rather than oxygen and the endogenous decay of cells was negligible. The overall cell balance about the fermenter is given as:

$$\frac{dX}{dt} = \mu X \tag{3}$$

μ (h^{-1}) is the specific growth rate. For the specific growth rate, the following kinetic models were be explored for their suitability in simulating the growth of *Aspergillus niger* cells during citric acid production.

Monod	Monod [16]	$\mu = \mu_{\max} \frac{S}{K_s + S}$	(4)
Haldane	Andrews [17]	$\mu = \mu_{\max} \frac{S}{K_s + S + (S^2 / K_I)}$	(5)
Hyperbolic	Novak et al.[18]	$\mu = \mu_{\max} \frac{S}{K_s + S} \frac{K_p}{K_p + P}$	(6)
Logistic equation	Baei et al. [19]	$\frac{dX}{dt} = \mu_{\max} X \left(1 - \frac{X}{X_{\max}}\right)$	(7)

μ_{max} (h^{-1}) is the maximum specific growth rate of biomass, K_s (g/L) is the substrate affinity constant, K_p (g/L) is the producat inhibition constant, K_i (g/L) is the substrate inhibition constant and X_{max} (g/L) is the maximum concentration of microbial cells.

2.5.3 Citric Acid Material Balance

For a batch operation, the rate of citric acid production in the fermenter is given as:

$$\frac{dP}{dt} = q_p X \quad (8)$$

In Equation (8), P (g/L)is the concentration of citric acid while the specific rate of citric acid production q_p (g citric acid/g biomass/h) is given by the Luedeking-Piret–like model presented in Equation (9).

$$q_p = Y_{p/x} \mu \quad (9)$$

$Y_{p/x}$ (g citric acid/g biomass) is the citric acid yield.

2.5.4 Energy Balance

The components of the general energy balance equation applied to a fermenter include energy generated by metabolism(Q_{met}), heat loss via by aeration($Q_{aeration}$), energy required for agitation(Q_{agit}), heat loss via evaporation(Q_{evap}), heat loss via convection (Q_{conv}), heat duty associated with feed inlet(Q_{feed}). The general energy balance equation as applied to a fermenter can then be expressed mathematically as:

$$\rho V C_p \frac{dT_b}{dt} = Q_{met} + Q_{aeration} + Q_{agit} + Q_{evap} + Q_{conv} + Q_{feed} \quad (10)$$

For a batch system without agitation, aeration and neglecting convective and evaporative losses, the general energy balance equation reduces to:

$$\rho_l V_l C_{pl} \frac{dT_b}{dt} = Q_{met} \quad (11)$$

The term on the left hand side is the energy accumulated in the system. The term on the right hand side is defined as follows.

$$Q_{met} = V_l \Delta H_{met} \quad (12)$$

Combining (12) with (11) results in:

$$\rho_l V C_{pl} \frac{dT_b}{dt} = V_l \Delta H_{met} \quad (13)$$

The metabolic heat ΔH_{met} is defined as:

$$\Delta H_{met} = \frac{\mu X}{Y_{x/s}} (\Delta H_{c.s} - Y_{x/s} \Delta H_{c.x}) \quad (14)$$

3. RESULTS AND DISCUSSION

3.1 Model Validation and Parameter Estimation

The batch fermenter model was validated against experimental data collected in the course of this work. This was done by estimating unknown model parameters. Table 1 shows the parameters estimated and their respective optimal estimate for the respective kinetic models considered.

Table 1: Values of estimated parameters using different models

Parameter	Optimal Estimate			
	Monod model	Haldane model	Hyperbolic model	Logistic equation
μ_{max} (h^{-1})	0.023	0.053	0.013	0.018
K_s(g/L)	1.622	5.000	0.001	N/A
$Y_{p/x}$(g/g)	14.099	12.855	13.671	14.300
$Y_{x/s}$(g/g)	0.074	0.077	0.071	0.076
K_I(g/L)	N/A	7.607	N/A	N/A
K_P(g/L)	N/A	N/A	0.257	N/A
P_m(g/L)	N/A	N/A	N/A	3.000
X_m(g/L)	N/A	N/A	N/A	0.445

Estimation of the maximum specific growth rate (μ_{max}) by the hyperbolic model and logistic equation resulted in fairly similar values. The Monod and Haldane models resulted in dissimilar values. For the substrate affinity constant (K_s), all the models gave very different results. Fairly similar values were obtained for the product and biomass yield for all models. These parameters were used to generate time profiles of substrate, microbial cells and citric acid concentrations for each model.

Figures 1 to 4 show the overlay plots which display the comparison between experimental and model predicted results for substrate (fermentable sugar), microbial cells and citric acid concentrations for Monod, Haldane, logistic and hyperbolic models respectively.

It was observed from Figure 1 that the Monod model was able to replicate the values of substrate and product concentrations fairly well but it performed poorly in predicting the concentration of microbial cells. Results obtained for the Haldane and logistic models as shown in Figures 2 and 3 showed that both models were able to predict the concentration of substrate fairly well but performed poorly in predicting the concentration of product and biomass. However, Figure 4 showed a high level of correlation between the experimental results and model predicted results obtained for the hyperbolic model. The model was able to replicate the concentrations of substrate, microbial cells and citric acid. This is an indication that the model exhibited a good fit with the experimental data.

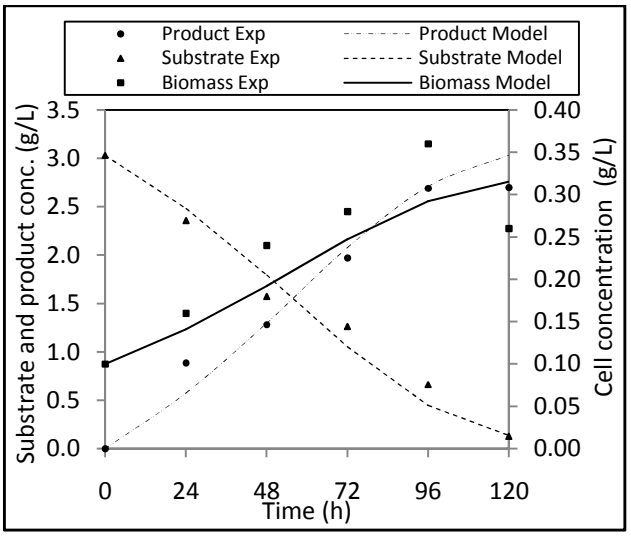

Figure 1: Comparison between experimental and model predicted results for Monod model

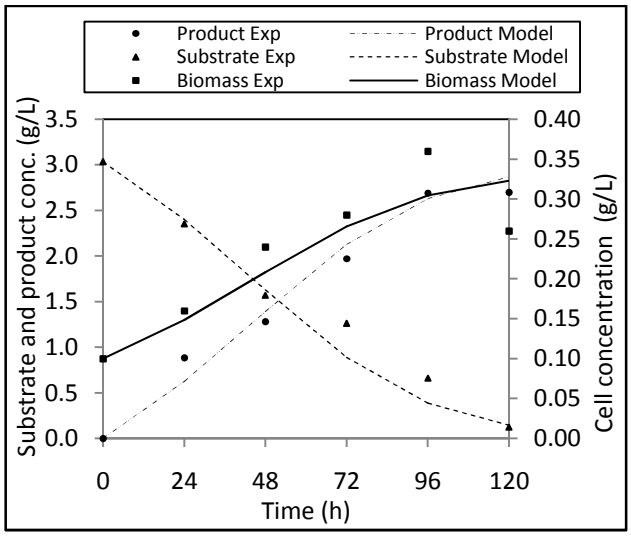

Figure 2: Comparison between experimental and model predicted results for Haldane model

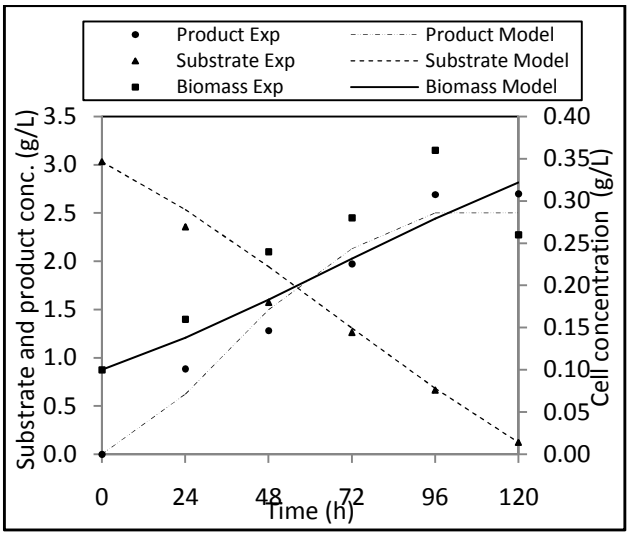

Figure 3: Comparison between experimental and the model predicted results for logistic equation

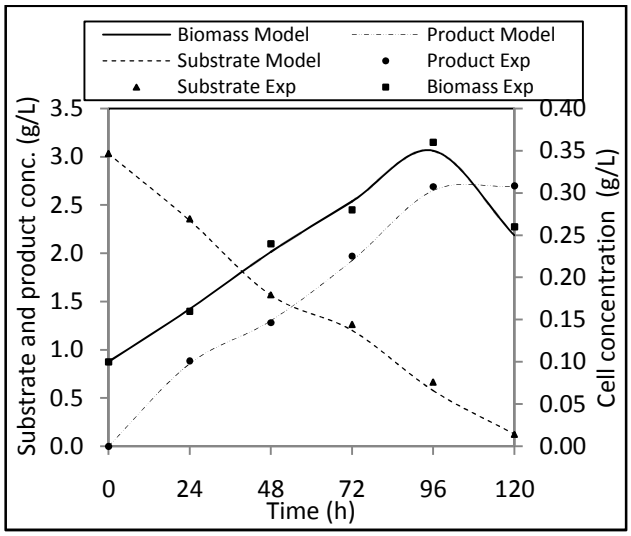

Figure 4: Comparison between experimental and model predicted results for hyperbolic model

3.2 Model Implementation

As a result of established validity of the Hyperbolic model (owing to the significant correlation between experimental and model predicted results), it was adopted for simulating the dynamic behaviour of the citric acid production process. The parameters estimated were used to generate time profiles for substrate, microbial cell and citric acid concentrations using the model. Figure 5 shows the time trajectory of substrate, microbial cell and citric acid concentrations in the course of the batch fermentation process. It was observed that there was a gradual and progressive reduction in the sugar substrate concentration from an initial value of 3.032g/Lat the start of fermentation to 0.152 g/Lat the end of fermentation corresponding to about 95% sugar substrate consumption. The reduction observed could be attributed to the metabolic utilisation of the sugar by the *Aspergillus niger*cells to produce citric acid. These results are in agreement with those reported by Al-Sheri and Mostafa, [20] for the production of citric acid from date palm syrup using immobilised *Aspergillus niger*cells. They recorded a progressive decrease in the concentration of sugar substrate in the course of fermentation with a maximum consumption capacity of about 80%. Baei et al.[19] also reported a similar decrease in the residual sugar content for citric acid production from apple pomace by *Aspergillus niger*. They attributed this observation to the formation of citric acid as a result of the metabolic consumption of the sugar substrate.

The concentration of *Aspergillus niger* cells increased from 0.10 g/Lat the start of fermentation to a maximum of 0.36 g/L at about 96hours indicating growth of the fermenting organism. Between 96 hours and 120hours, there was a reduction in the concentration of *Aspergillus niger* cells 0.26 g/L. The decline observed could be as a result of substrate limitation and also probably due to the accumulation/presence of toxic substances in the fermentation vessel that might inhibit the action of the fermenting organism. The decline in growth of microbial cells could also be as a result of the inhibitory effects presented at high concentrations of citric acid, decay in the enzyme system responsible for biosynthesis of citric acidand reduction in the amount of important nutrients such as Nitrogen and Phosphorus [20-22]. The parameters for *Aspergillus niger* growth μ_{max}, $Y_{x/s}$ and K_s as calculated by the hyperbolic model were 0.013h^{-1}, 0.071 g/g and 0.001 g/L respectively as shown in Table 1.

Figure 5 showed that the bioproduction of citric acid was almost linear with respect to cell growth from the start of fermentation till about 96 hours. This is an indication that citric acid formation is growth associated. The concentration of citric acid produced increased steadily from zero to about 2.7 g/L. Increasing the fermentation time beyond 96 hours had no positive influence on citric acid production. Since the formation of citric acid is growth associated, the decrease in citric acid productivity observed could be attributed to the decline in the growth of *Aspergillus niger* cells observed at 96 hours. These observations were also in agreement with those of Nadeem et al. [2]. They investigated the enhancement of citric acid production using low molecular weight alcohol and recorded similar reductions in citric acid production after attaining a maximum value. The parameters for citric acid production $Y_{p/x}$ and K_p as calculated by the hyperbolic model were 13.671 g/g and 0.257 g/L respectively.

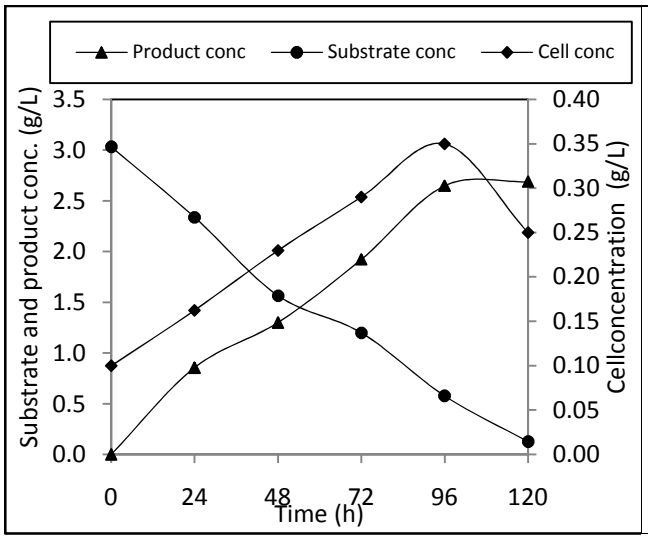

Figure 5: Comparison between experimental and the model predicted results for hyperbolic model

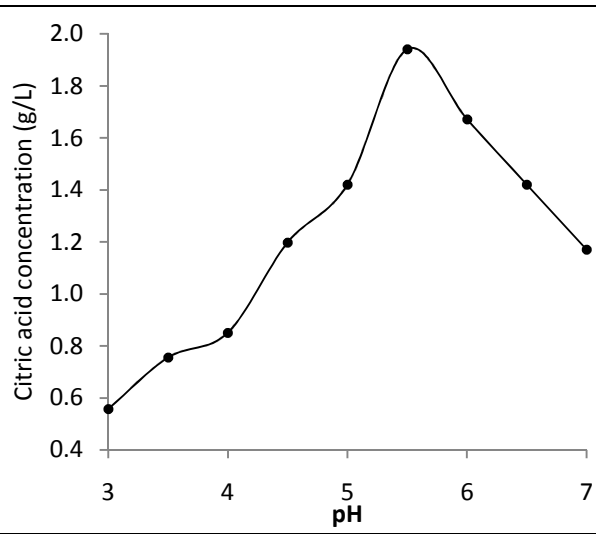

Figure 6: Variation of citric acid concentration with pH

4.2.1 Effect of pH on Citric Acid Production

The effect of pH on the production of citric acid is presented in Figure 6. It was observed that the concentration of citric acid increased with pH up to a maximum at a pH of 5.5 after which it declined. The results show that the best citric acid concentration of 1.94 g/L was obtained at a pH of 5.5.The results obtained are in agreement with those reported by Al-Sheri and Mostafa, [20] as well as Kahlon et al. [23]. Both set of researchers reported an optimum pH of 5.5 for citric acid production from date palm syrup and sugar cane molasses respectively. The pH is important in two respects. Firstly, spore germination which is required for fermentation requires a pH of 5 and above to occur. Secondly, protons are released when ammonia is absorbed by germinating spores. This causes a release of hydrogen ions thus lowering the pH of the medium. The low pH has the effect of improving citric acid production and providing a close to sterile environment which reduces the risk of contamination [5]. Operating at very high pH values results in the accumulation of unwanted products such as oxalic acid [20]

3.2.2 Effect of Initial Sugar Concentration on Citric Acid Production

Figure 7 shows the effect of initial sugar concentration on citric acid production. Citric acid concentration increased with initial sugar concentration up to a maximum of 2.33 g/L at an initial sugar concentration of 40 w/v. Beyondan initial sugar concentration of 40 w/v, there was a decline in the concentration of citric acid. The initial sugar concentration is a very important parameter in citric acid fermentation. Al-Sheri and Mostafa, [20] reported that the advantages presented by high concentrations of fermentable sugars in the fermentation medium may be the suppression of osmo-sensitive contaminants and reduced dilution requirements. However, Hossain et al. [24] reported that increasing the sugar concentration beyond the optimum could lead to the repression of the enzyme keto-glutarate dehydrogenase which subsequently leads to a decline in citric acid productivity.

Pazouki et al. [6] reported that using lower concentrations of sugar substrate leads to the accumulation of oxalic acid in the culture medium which reduces the yield of citric acid. Xu et al. [25] studied the effect of sugar substrate (maltose, sucrose, glucose, mannose and fructose) concentration on citric acid productivity in submerged fermentation. They observed that the highest yields of citric acid were recorded at sugar concentration of 10% w/v for all sugars studied with the exception of glucose where 7.5% gave the best results. They further noted that no citric acid was produced in fermentation medium when the concentration of sugar substrate was less than 2.5%. This shows the importance of identifying the optimum sugar concentration for citric acid production as operating below or above the optimum will result in unfavourable citric acid productivity [5].

3.2.3 Effect of Temperature on Citric Acid Production

The effect of temperature on the production of citric acid is illustrated in Figure 8. There was a positive relationship between citric acid production and temperature up to 30°C. Beyond30°C, there was an observable decline in citric acid production.

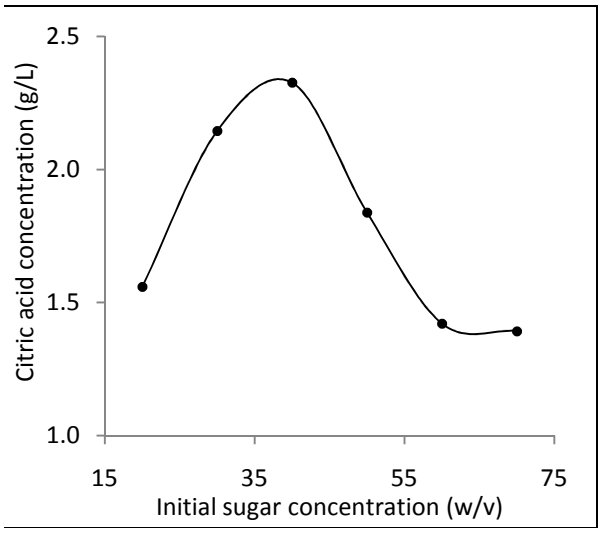

Figure 7: Variation of citric acid concentration with initial sugar concentration

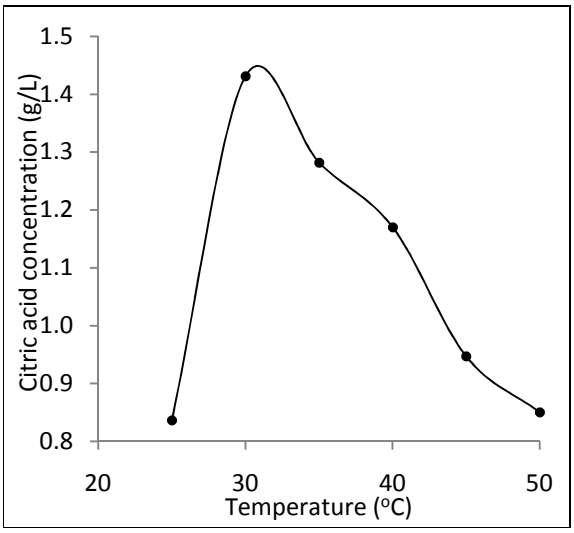

Figure 8: Variation of citric acid concentration with temperature

The decline observed beyond 30°C might have been due to the denaturation of the enzyme citrate synthase responsible for citric acid production and the possible accumulation of toxic products in the fermentation vessel. The fermentation temperature is important in that when cells are grown under non ideal temperature conditions, they exhibit signs of adverse growth and metabolic production [26]. Arzumanov et al. [22] and Nampoothiri et al.[27]reported that citric acid production could be affected by a slow germination of the fungi, slow metabolic activity and reduced cell viability when *Aspergillus niger* cells are incubated under low temperatures.The results obtained are in agreement with those reported by previous researchers [20,22,28]. The optimum temperature obtained in this study is in agreement with the fact that filamentous fungi such as *Aspergillus niger* are mesophilic thus requiring optimal temperatures between 25°C and 35°C for growth [29,30].

4. CONCLUSION

The modelling and simulation of the batch production of citric acid from corn starch hydrolysate was carried out in this study. A validated hyperbolic model which incorporates a product inhibition term was able to predict the time trajectory of sugar substrate, microbial cell and citric acid concentration in the batch fermenter to a relatively high level of confidence. The production of citric acid is associated with the growth of *Aspergillus niger* cells. The optimum pH, initial sugar concentration and temperature for citric acid fermentation were 5.5, 40w/v and 30°C respectively. These were the conditions at which the best citric acid production was recorded.

REFERENCES

[1] Amenaghawon, N.A, Nwaru, K.I., Aisien, F.A., Ogbeide, S.E. and Okieimen, C.O. (2013). "Application of Box-Behnken Design for the Optimization of Citric Acid Production from Corn Starch Using Aspergillusniger".*British Biotechnology Journal*, Vol. 3, Number 3, 2013, pp. 236-245.

[2] Nadeem, A., Syed, Q., Baig, S., Irfan, H. and Nadeem, M. "Enhanced production of citric acid by Aspergillus niger M-101 using lower alcohols". *Turkish Journal of Biochemistry*, Vol. 35, Number 1, 2010, pp. 7–13.

[3] Lancini, G. *Parte I - L'usoindustrialedeimicrorganismi. Storia e campi di applicazione. In: Donadio, S.; Marino, G. (eds.). BiotecnologieMicrobiche*. Milan: Casa EditriceAmbrosiana. 2008.

[4] Papagianni, M. "Advances in citric acid fermentation by Aspergillus niger: Biochemical aspects, membrane transport and modelling". *Biotechnology Advances*, Vol. 25, Number 3, 2007, pp. 244-263.

[5] Max, B., Salgado, J.M., Rodríguez, N., Cortés, S., Converti, A. and Domínguez, J.M. (2010). "Biotechnological production of citric acid". *Brazilian Journal of Microbiology*, Vol. 41, Number 4, pp. 862-875.

[6] Pazouki M., Felse P.A., Sinha H. and Panda T. "Comparative studies on citric acid production by *Aspergillusniger*and Candida lipolytica using molasses and glucose". *Bioprocess Engineering*, Vol. 22, 2000, pp. 353–361.

[7] Vandenberghe, L.P.S., Soccol, C.R., Pandey, A. and Lebeault, J.M. "Solid-state fermentation for the synthesis of citric acid by *Aspergillusniger"*. *Bioresource Technology*, Vol. 74, 2000, pp. 175–178.

[8] Amenaghawon, N. A. and Okieimen, C.O. (2011). "Dynamic modeling, simulation and optimisation of an isothermal CSTR for methanol synthesis. *Journal of Nigerian Society of Chemical Engineers*, Number 26, Number 2, pp. 36-47.

[9] Suja M.R.M. and Thyagarajan, T. "Modelling of continuous stirred tank reactor using artificial intelligence techniques". *International Journal of Simulation and Modelling*, Vol. 8, Number 3, 2009, pp. 145–155.

[10] Andres, H., Hu, Z., Snowling, S. and Schraa, O. "Model-Based Optimum Design of Sequencing Batch Reactors for COD and Nitrogen Removal from a Slaughterhouse Wastewater". *Proceedings of the Water Environment Federation*, Vol. 2006, Number 7, 2006, pp. 5100-5107.

[11] Lee, T.T., Wang, F.Y. and Newell, R.B. "A generalised procedure for modelling and simulation of activated sludge plant using lumped-parameter approach". *Journal of Environmental Science and Health. Part A: Environmental Science and Engineering and Toxicology*, Vol. 32, Number 1, 1997, pp. 83-104.

[12] Lotfy, W.A., Ghanem, K.M. and El-Helow, E.R."Citric acid production by a novel Aspergillusniger isolate: I. Mutagenesis and cost reduction studies". *Bioresource Technology*, Vol. 98, Number 18, 2007, pp. 3464-3469.

[13] Miller, G.L. "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar". *Analytical Chemistry*, Vol. 31, Number 3, 1959, pp. 426-428.

[14] Marier, J.R. and Boulet, M. "Direct Determination of Citric Acid in Milk with an Improved Pyridine-Acetic Anhydride Method". *Journal of Dairy Science*, Vol. 41, Number 12, 1958, pp. 1683-1692.

[15] Pirt, S.J."The Maintenance Energy of Bacteria in Growing Cultures. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, Vol. 163, Number 991, 1965, pp. 224-231.

[16] Monod, J. "The Growth of Bacterial Cultures". *Annual Review of Microbiology*, Vol. *3,* Number 1, 1949, pp. 371-394.

[17] Andrews, J."A mathematical model for the continuous culture of microorganisms utilising inhibitory substances". *Biotechnology and Bioengineering*, Vol. 11, 1968, pp. 478-483.

[18] Novak, M., Strehaiano, P., Moreno, M. and Goma, G. "Alcoholic fermentation: On the inhibitory effect of ethanol". *Biotechnology and Bioengineering*, Vol. *23,* Number 1, 1981, pp. 201-211.

[19] Baei, M. S., Mahmoudi, M. and Yunesi, H. "A kinetic model for citric acid production from apple pomace by Aspergillus niger". *African Journal of Biotechnology*, Vol. 7*,* Number 19, 2008, pp. 3487-3489.

[20] Al-Sheri M.A, and Mostafa, Y.S. "Citric acid production from date syrup using immobilized cells of *Aspergillusniger"*. *Biotechnology*, Vol. 5, Number 4, 2006, pp. 461-465.

[21] Alvarez-Vasquez, F., Gonzalez, A.C. and Torres, N.V. "Metabolism of citric acid production by *Aspergillusniger*: model definition, steady-state analysis and constrained optimization of citric acid production rate". *Biotechnology and Bioengineering*, Vol. 70, 2000, pp. 82-108.

[22] Arzumanov, T.E., Shishkanova, N.V. and Finogenova, T.V. "Biosynthesis of citric acid by *Yarrowa lipolytica* repeat batch culture on ethanol". *Applied Microbiology and Biotechnology,* Vol. 53, 2000, pp. 525-529.

[23] Kahlon, S.S., Arora, M. and Kaur, J."Optimization of parameters for production of citric acid by immobilized Aspergillusniger". *Journal of Research Punjap Agricultural University*, Vol. 29, 1992, pp. 359-366.

[24] Hossain, M., Brooks, J.D. and Maddox, I.S. (1984). "The effect of the sugar source on citric acid production by Aspergillusniger". *Applied Microbiology and Biotechnology,* Vol. 19, Number 6, 1984, pp. 393-397.

[25] Xu, D.B., Madrid, C.P., Röhr, M. and Kubicek, C.P."The influence of type and concentration of the carbon source on production of citric acid by *Aspergillusniger"*. *Applied Microbiology and Biotechnology*, Vol. 30, Number 6, 1989, 553-558.

[26] Ellaiah, P., Srinivasulu, B. and Adinarayana, K. "Optimisation studies on neomycin production by a mutant strain of Streptomyces marinensis in solid state fermentation". *Process Biochemistry*, Vol. 39, Number 5, 2004, pp. 529-534.

[27] Nampoothiri, M.K., Baiju, T.V., Sandhya, C., Sabu, A., Szakacs, G. and Pandey, A. "Process optimization for antifungal chitinase production by *Trichodermaharzianum". Process Biochemistry*. Vol. 39, Number 11, 2004, pp. 1583-1590.

[28] Ali, S., Haq, I.U., Qadeer, M.A. and Iqbal, J. Production of citric acid by Aspergillusniger using cane molasses in a stirred fermentor. *Electronic Journal of Biotechnology*, Vol. 5, Number 3, 2002, pp. 19-20.

[29] Reid, I.D."Solid-state fermentation for biological delignification". *Enzyme and Microbial Technology*, 11, 1989, pp. 786-803.

[30] Suresh, P.V. and Chandrasekaran, M. "Impact of process parameters on chitinase production by an alkalophilic marine *Beauveriabassiana* in solid state fermentation". *Process Biochemistry*, Vol. 34, 1999, pp. 257–267.