Full Paper

OPTIMIZATION OF BREADFRUIT HYDROLYSATE MEDIUM FOR GLUCONIC ACID PRODUCTION BY FILAMENTOUS FUNGUS *ASPERGILLUS NIGER*

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ABSTRACT

Medium composition optimization for the growth of the *Aspergillus niger* on breadfruit starch hydrolysate and its gluconic acid production ability was carried out at shake flask level using Graeco-Latin square design method. The effect of carbon concentration, nitrogen source and pH were evaluated. From the experiments, results of medium (M8) with 100g/l of breadfruit hydrolysate, urea as the nitrogen source and pH value of 5.5 was found to be the optimum medium. The gluconic acid concentration obtained from this medium was 97.20 g/l, which gave the highest conversion of breadfruit starch hydrolysate to gluconic acid (97%). The results indicated that pH value of 5.5 favours the production of gluconic acid better than pH of 4.5 and 6.5. The nitrogen source of L-glutamic acid and urea encouraged more accumulation of biomass concentration compared to ammonium sulphate. Kinetic studies of data obtained for medium M8 gave P_t of 97.20 g l^{-1} (Y'_{ps} of 0.960 g g^{-1} and 90%Y_{acid}), Q of 0.024 g g^{-1} d^{-1}, Q_p of 25.01 g l^{-1} d^{-1}, Q_s of 27.41 g g^{-1} d^{-1} and Y'_{xs} of 0.375 (μ of 0.11 h^{-1}). This study showed that the cheap crop – breadfruit can be used as the sole carbon source for gluconic acid production. The results obtained can be scaled up for a pilot or industrial scale plant.

Keyword: *Breadfruit, hydrolysis, gluconic acid, Aspergillus niger, fermentation*

1. INTRODUCTION

D-gluconic acid, also known as pentahyroxycaproic acid, is an oxidation product of D-glucose catalysed by glucose oxidase (Mukhopadhyay et al., 2005; Ramachandran et al., 2006). Various methods exist for the gluconic acid production viz. chemical, electrochemical, biochemical and bioelectrochemical (Dirkx and van der Baan, 1981; Laane et al., 1984; Rao and Panda, 1993). Demand for this acid is met mainly via fermentation (Shah and Kotharis, 1991; Bao et al., 2001; Liu et al., 2003). Gluconic acid and its salts are important materials widely used in food, pharmaceutical, detergent, leather and photographic industries (Das and Kundu, 1987; Hustede et al., 1898). It is also used as an additive to improve cement hardening (Magnuson, J. and Lasure, 2004).

Due to enormous demand of about 60,000 tons per annum, the microbial fermentation processes are exclusively used for commercial gluconic acid production using glucose as the major carbon source (Röhr et al., 1996; Roukas, 2000; Singh et al., 2003). A variety of carbohydrates have been used for gluconic acid production. It has been suggested that the economics of fermentation of gluconic acid can be improved by using cheap raw materials, provided the producing microorganism can metabolise the particular carbohydrate (Mukhopadhyay et al., 2005). Singh et al. (2005) successfully used some agro-food by products (grape must, banana must and sugarcane molasses) for gluconic acid. Vassilev et al. (1993) used hydrol (corn starch hydrolysate) as fermentable sugar to produce gluconic acid while Rao and Panda (1994) employed Indian cane molasses. Ikeda et al. (2006) produced the acid using saccharified waste paper with glucose as carbon source.

Breadfruit (*Artocarpus communis*) which is native to Malaysia was introduced to Ifewara, Southwestern Nigeria from the Caribbeans and spread to the neighbouring villages. It is regarded as poor man's substitute for yam in this part of Nigeria (Adewusi et al., 1995). But elsewhere it is used in a variety of food preparations such as cakes, syrup, jam, cooked into puddings or baked, roasted or fried as chips (Weir, 1982). However, the major factor militating against its utilisation as a staple food in most tropical regions is deterioration during fruit development (Omobuwajo and Wilcox, 1989; Adewusi et al., 1995). The starch content of unripe mature breadfruit pulp is 77% (Adewusi et al., 1995). The breadfruit has been made into flour and evaluated in bakery products (Olatunji and Akinrele, 1978). Solomon et al. (1994) and Betiku and Ajala (2010) carried out hydrolysis studies on its starch flour and used hydrolysate for ethanol and gluconic acid production, respectively as a way of adding value to the fruit.

The aim of this work was to study the gluconic acid production using *A. niger* grown on glucose-rich syrup obtained from breadfruit starch. Furthermore, optimization of the media was carried out by investigating the effects of pH, nitrogen source and sugar concentration on the gluconic acid production. The pertinent kinetic parameters were also determined.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Breadfruit

Freshly harvested mature but unripe fruits from breadfruit tree were obtained from Ile-Ife, Osun State, Nigeria. The breadfruits were washed in clean water to remove adhering latex and dirt. They were manually peeled and sliced to pieces with a stainless steel knife. The sliced fruits were sun-dried to constant weight and milled to flour.

2.1.2 Enzymes

Alpha-amylase (E.C.3.2.1.1) from bacterium source (*Bacillus licheniformis*) and glucoamylase (E.C.3.2.1.3) from *Aspergillus niger* were both obtained from the Federal Institute of Industrial Research, (FIIRO), Oshodi, Lagos, Nigeria.

2.2 Glucose syrup production

The method of Betiku and Ajala (2010) was employed for the hydrolysis. The flour obtained was made into starch slurry by adding appropriate quantity of water. To make 10% slurry, 10 g of flour was weighed into 100 ml distilled water to make slurry. The solution of 40ppm Ca^{2+} was added for the stability of the enzyme. The pH was adjusted to 6.5 with Citrate-phosphate buffer. Gelatinization was done by heating the mixture to 97°C and was held at this temperature for 10 min. The slurry was cooled to 60°C. The gelatinized starch slurry was liquefied by adding 2% (w/v) of α-amylase at 60°C for a period of 40 min. The Liquefied starch was buffered to pH of 4.5 and was subsequently saccharified with 2% (w/v) glucoamylase incubated at 50°C for 70 min. The enzyme activity was stopped by heating the mixture 100°C for 15 min.

2.3. Microorganism and Medium Composition

2.3.1 Microorganism

A pure culture of *Aspergillus niger* strain provided by the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria was used throughout this study. Organism was maintained as direct stock culture from which inocula were prepared. Spore formation was carried out on potato dextrose agar at 30°C for 6-7 days and then the spores were stored in a 4°C refrigerator with regular sub-culturing.

2.3.2 Medium Composition

The preculture medium was composed of in g/l, glucose, 50; yeast extract, 3; malt extract, 3; polypeptone, 5; calcium carbonate, 20. Production medium was composed (g/l) of carbon source, 50, 100 or 120; Ammonium sulphate, L-glutamic acid or Urea 1.35; Na_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.15; Calcium carbonate, 60 (Sakurai et al., 1989). The carbon source for production medium was glucose rich syrup obtained as breadfruit hydrolysate. All media and flasks were sterilized using an autoclave at 121°C for 20 min.

2.4. Medium Optimization for Gluconic Acid Production.

The 3 x 3 Graeco-Latin square design was used for the optimization of the studies. Three different concentrations of breadfruit hydrolysate such as 50 g/l, 100 g/l and 120g/l, nitrogen sources: ammonium nitrate, L-glutamic acid and urea, and pH of 4.5, 5.5 and 6.5 were varied and compared in a single experiment (Table 1).

2.5. Inoculum preparation

Five millilitres of the aseptically harvested spores from the sporulating surface with 50 ml sterile water were inoculated into 250 ml Erlenmeyer flasks containing 50 ml of preculture medium composition. The inoculated flasks were shaken continuously on an environment-controlled incubator shaker manufactured by New Brunswick Scientific Co. (USA) at 200 rpm and 30°C for 18 h before it was used for the fermentation process.

2.6. Shake Culture Experiment

For the gluconic acid production, 5 ml of preculture medium was inoculated into 250 ml Erlenmeyer flasks containing 50 ml of production medium. The cultivation was carried out at 30°C at 200 rpm in an environment-controlled incubator shaker for 72 h. Samples were withdrawn at regular intervals for gluconic acid concentration analysis. Samples were also collected and filtered. The residue was processed for biomass and the clear supernatants were used for the reducing sugar analysis.

2.7. Analytical methods

2.7.1 Gluconic acid concentration

A millilitre of sample from medium was mixed with 1 ml of fresh broth without calcium carbonate. Thereafter, 4 ml of 0.1 M of Ammonium oxalate ($(NH_4)_2C_2O_4.H_2O$) was added with 2 ml of

Table 1: Medium Optimization for Gluconic Acid Production using Graeco – Latin Square Design

Ing.	Ing. type	M1	M2	M3	M4	M5	M6	M7	M8	M9
Bread Fruit	50g/l	50g/l			50g/l			50g/l		
	100g/l		100g/l			100g/l			100g/l	
	120g/l			120g/l			120g/l			120g/l
Nitrogen 1.35g/l	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$						
					L-glu	L-glu	L-glu			
								Urea	Urea	Urea
pH	4.5	4.5			4.5			4.5		
	5.5		5.5			5.5			5.5	
	6.5			6.5			6.5			6.5

Key: Ing. –Ingredient, M – Medium

1/50 N ammonium hydroxide (NH_4OH) to the initial mixture. The mixture was then boiled for 10 minutes. Afterwards, it was cooled to room temperature. The mixture was centrifuged at 8000 g for 5 min and the supernatant was removed. The resulting precipitate was washed with distilled water for three times and dissolved by heating in the presence of 6 ml of 2 N Hydrosulphuric acid (H_2SO_4). The final solution was titrated against N/10 Potassium permanganate, $KMnO_4$ (Ikeda et al., 2006).

2.7.2 Reducing sugar concentration Assay

The DNS method described by Miller (1959) and modified DNS Reagent was used to determine the reducing sugar concentration in this work. The fermented broths were collected and 3 ml of the DNS solution was added in the test tubes and was boiled for 15 min, cooled and diluted appropriately after which their absorbencies were measured at a wavelength of 540 nm using the UV-Visible Spectrophotometer. Readings were interpreted with a calibration curve prepared by adding DNS solution to increasing concentration of glucose.

2.7.3 Biomass concentration determination

The filter paper was preweighed before it was used to filter the broth. The filtered mycelia mat was washed with acidified (4 mol l^{-1} hydrochloric acid) double distilled water to convert the insoluble calcium carbonate to soluble calcium chloride. The separated mycelia mat was washed several times with deionized water until pH of washing was neutral (7.0). The residue was oven dried at 80°C for 24 h to constant weight. After which it was allowed to cool and final weight was recorded. The weight of the biomass was determined by subtracting the weight of the filter paper from the weight of the paper plus the cells (Singh and Singh, 2006).

2.8. Kinetic Data Analysis

Kinetic analysis of the data from shake cultures was determined as described by Doran (1994). Polymath Software and Microsoft Excel were employed in determination of the various parameters measured for this kinetic study.

3. RESULTS AND DISCUSSION

3.1. Breadfruit hydrolysate utilization

The study investigated the possibility of using Breadfruit starch hydrolysate as the sole carbon source for the production of gluconic acid using *Aspergillus niger* under shake culture cultivation. Figures 1-3 show the profiles of gluconic acid, reducing sugar and biomass concentrations against fermentation time using 50 g/l, pH of 4.5 while varying the nitrogen source. The results showed that *Saccharomyces cerevisiae* was able to metabolize the Breadfruit starch hydrolysate for growth and product formation. The microorganism consumed about 90% of the hydrolysate within 24 h of cultivation irrespective of the nitrogen source employed. By 36 h of cultivation, the hydrolysate had been completely utilized. The profile for biomass concentration against time was the same for the three cases considered. The *A. niger* cells grew well in all the media as exemplified in increase in the biomass concentration from the initial phase until 48th hour of the cultivation before it declined slightly. The peak observed ranged from 19.76 to 22.07 g/l for the three media (Figures 1-3). There was a decline in all the cases afterwards. This may be attributed to the hydrolysate that has been depleted.

Gluconic acid formation increased from the beginning of the cultivation up until the hydrolysate was completely exhausted in the case of the media with L-glutamic acid and urea as nitrogen sources.

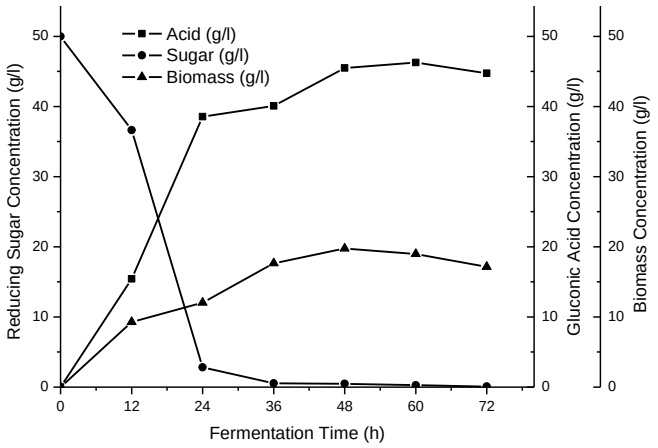

Figure 1: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 50 g/l of Breadfruit Hydrolysate and Ammonium sulphate at pH of 4.5

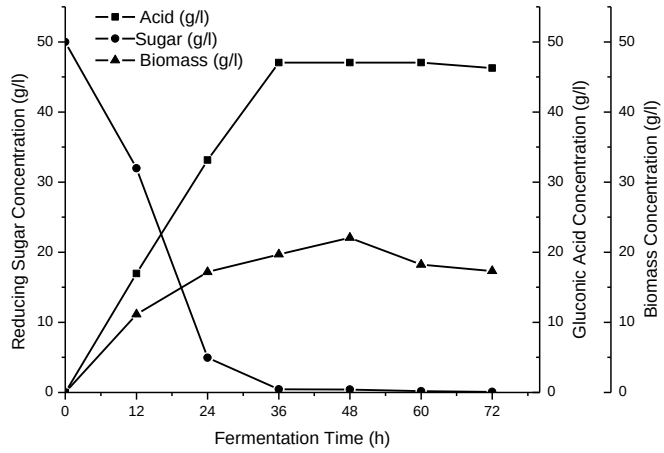

Figure 2: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 50 g/l of Breadfruit Hydrolysate and L-glutamic acid pH of 4.5

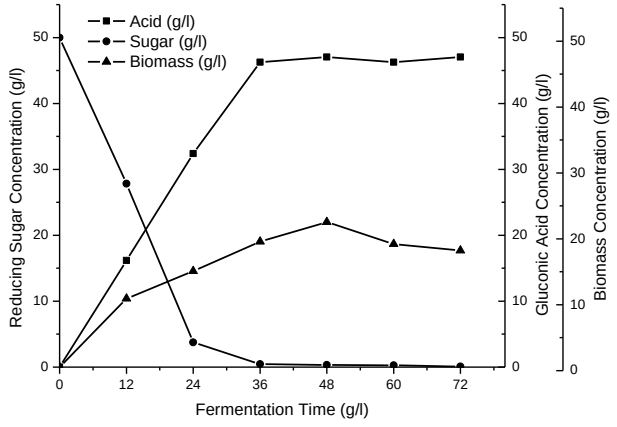

Figure 3: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 50 g/l of Breadfruit Hydrolysate and Urea at pH of 4.5

The production was stationary from this point to the end of the investigation. This observation suggests that there was a direct correlation between the Breadfruit starch hydrolysate consumption and the gluconic acid formation. Noteworthy, is the fact that the product did not decline after the hydrolysate had been completely used up. In the medium with ammonium sulphate as nitrogen source, gluconic acid production increased throughout the period of observation.

The gluconic acid yield was between 46.26 and 46.72 g/l for the three media considered. Ikeda et al. (2006) produced 46.0 and 40.4 g/l gluconic acid from 50 g/l of hydrolysed waste paper and synthetic glucose, respectively. The corresponding yields of gluconic acid reported by same authors, based on glucose consumption were 92 and 80%, respectively. In this study, the gluconic acid yield based on Breadfruit starch hydrolysate consumption (50 g/l) at pH of 4.5 using $(NH_4)_2SO_4$, L-glutamic acid and urea were 79, 85 and 87%, respectively.

The results obtained when Breadfruit starch hydrolysate concentration was increased to 100 g/l and pH of 5.5 with varying nitrogen sources was used are depicted in Figures 4-6. The results showed similar profiles with those obtained for media with 50 g/l Breadfruit starch hydrolysate and pH of 4.5 (Figures 1-3).

The yeast showed no problem metabolizing the hydrolysate for growth and gluconic acid formation. More than 90% of the hydrolysate had been consumed 48 h into the cultivation. It was observed that as the hydrolysate was being consumed there was a corresponding increase in the amount of the gluconic acid produced. The media with urea as nitrogen source produced the highest amount of gluconic acid after 72 h of cultivation. The amount of gluconic acid produced in all the three cases double the amount observed when 50 g/l of the Breadfruit starch hydrolysate was employed.

As noted earlier when 50 g/l of the hydrolysate was used, there was a direct relationship between the hydrolysate consumption and the quantity of gluconic acid produced. The biomass concentration production increased almost linearly from the initial phase and reached a maximum after 48 h of cultivation (Figures 4-6). This observation was similar to the results obtained in studies with 50 g/l of Breadfruit starch hydrolysate. The biomass concentrations were double the amount produced when media with 50 g/l breadfruit starch hydrolysate were employed. The peak biomass concentration ranged between 39.42 to 45.60 g/l for the media with 100 g/l Breadfruit starch hydrolysate and pH of 5.5 (Figures 4-6). The gluconic acid yield based on Breadfruit starch hydrolysate consumption (100 g/l) at pH of 5.5 using $(NH_4)_2SO_4$, L-glutamic acid and urea were 88, 89 and 90%, respectively.

The Breadfruit starch hydrolysate was increased to 120 g/l and pH adjusted to 6.5 using the three nitrogen sources. The results obtained are presented in Figures 7-9.

The hydrolysate utilization profile was similar to the earlier results reported for 50 and 100 g/l. After 48 h of cultivation, almost all the hydrolysate had been consumed irrespective of the nitrogen source used. Gluconic acid concentration increased throughout the period of observation. The highest concentration (108.71 g/l) of the acid was observed at the 72nd hour with the medium with urea as nitrogen source. Although the microorganism showed not problem metabolizing the Breadfruit starch hydrolysate, the gluconic acid produced was not commensurate to the hydrolysate consumed. This observation may be due to the pH of the medium (6.5).

The biomass concentration profile was similar to the results presented in Figures 1-6. The peak was observed at 48 h of cultivation. There was no corresponding increase in the biomass concentration when the hydrolysate concentration was increased from 100 to 120 g/l in all media investigated. The pH used may have affected the growth of the yeast and subsequently the amount of acid produced. The medium with urea as nitrogen source did best in terms of the gluconic acid and biomass concentrations. The gluconic acid yield based on Breadfruit starch hydrolysate consumption (120 g/l) at pH of 6.5 using $(NH_4)_2SO_4$, L-glutamic acid and urea were 76, 70 and 82%, respectively.

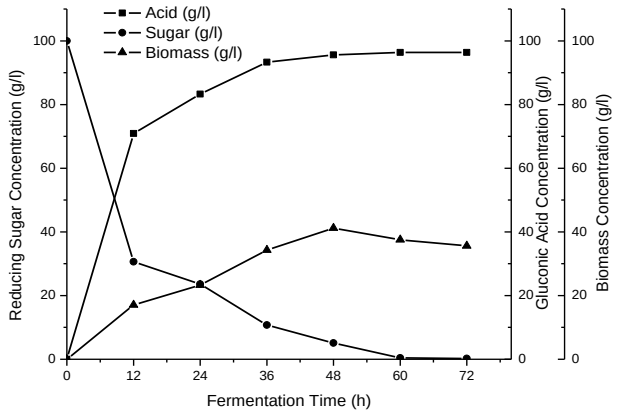

Figure 4: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 100 g/l of Breadfruit Hydrolysate and Ammonium sulphate at pH of 5.5

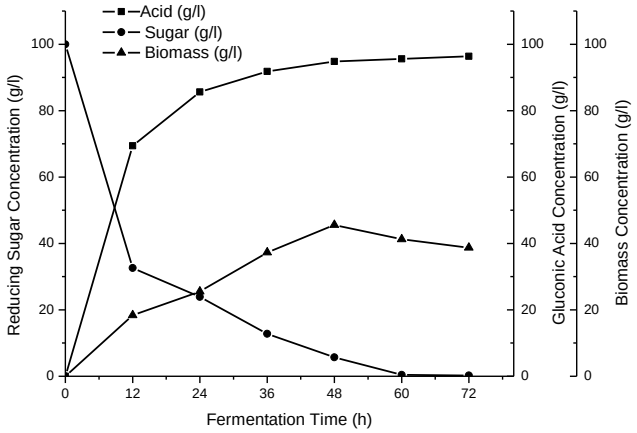

Figure 5: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 100 g/l of Breadfruit Hydrolysate and L-glutamic acid sulphate at pH of 5.5

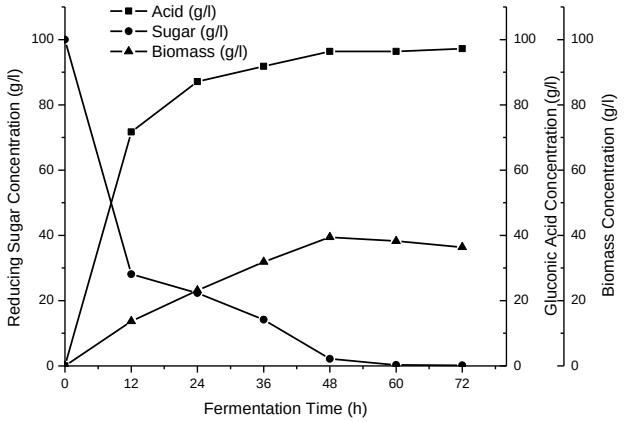

Figure 6: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 100 g/l of Breadfruit Hydrolysate and Urea at pH of 5.5

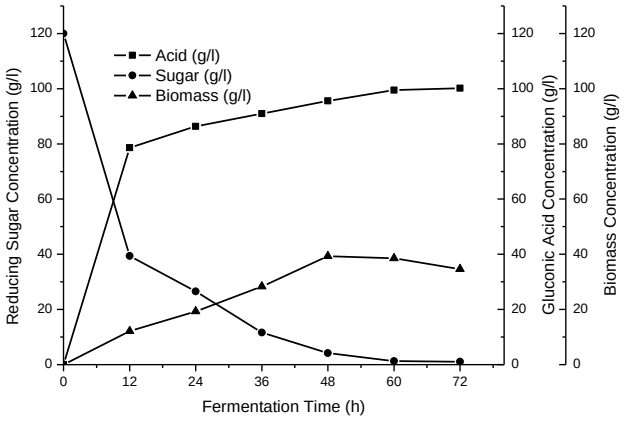

Figure 7: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 120 g/l of Breadfruit Hydrolysate and Ammonium sulphate at pH of 6.5

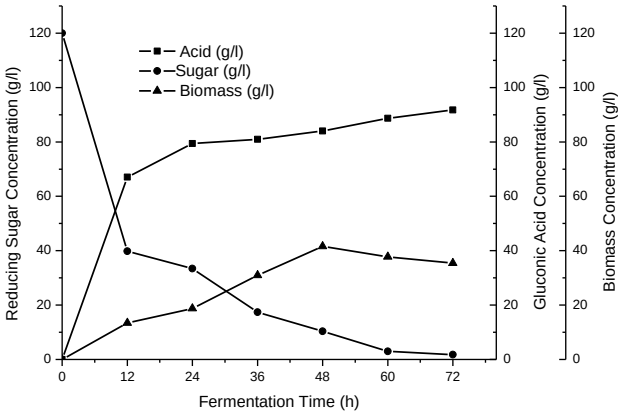

Figure 8: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 120 g/l of Breadfruit Hydrolysate and L glutamic acid at pH of 6.5

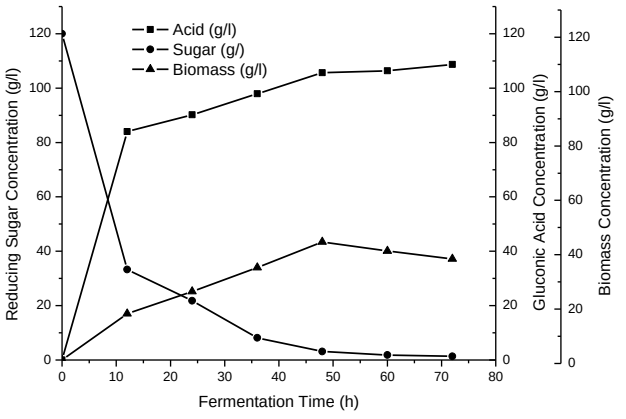

Figure 9: Plot of Reducing Sugar, Gluconic Acid and Biomass Concentrations against Fermentation Time for Medium with 120 g/l of Breadfruit Hydrolysate and Urea at pH of 6.5

Generally, the Breadfruit starch hydrolysate used in this study as the sole carbon source for the cultivation of *A. niger* gave promising results. The results strongly suggest that the hydrolysate does not show any negative effect on the gluconic acid production and hence could be used as an alternative carbon source. pH is an important parameter that influences gluconic acid production (Ramachandran et al., 2006). The pH values employed in this study were chosen based on the literature reports. The pH range for gluconic acid production is 4.5 to 6.5 (Röhr et al., 1983). Ikeda et al. (2006) used pH 5-6 for their study. Lui et al. (2003) and Mukhopadhyay et al. (2005) set the pH of the media used for gluconic acid production at 6.0. Singh and Singh (2006) reported high gluconic acid production when pH range of 4.5-6.5 was used. They reported that gluconic acid production beyond this range of pH was substantially low. Znad et al. (2004) observed pH of 5.5 as optimum for *A. niger*. In this work, media with pH of 5.5 gave higher yields of gluconic acid compared to pH of 4.5 and 6.5. The effect of nitrogen source on the gluconic acid production was also investigated. It has been reported that very low concentration is needed for optimal gluconic acid production (Röhr et al., 1983). Three different nitrogen sources investigated showed that they all supported the acid production. However, urea gave the best result.

3.2 Kinetics Studies

The results of kinetics on the data collected from shake flask experiments are presented in the Table 2. The highest final concentration of gluconic acid (P_t) of 108.71g/*l was obtained from medium M9* and the lowest value of 44.72 g/l was observed in medium M1. Also the highest *percentage yield of theoretical* (Y_{acid}) *of* 90 was obtained from medium M8 while t*he lowest* Y_{acid} of 70 was obtained from medium M6. The extent to which the microorganism was able to utilize the Breadfruit hydrolysate as the sole carbon was estimated as the specific growth rate (μ) and growth yield coefficient (Y'_{xs}). The Y'_{xs} obtained at media M1, M2 and M3 were 0.314, 0.380 and 0.317 g cell g^{-1}, respectively while μ ranged between 0.003 and 0.014 h^{-1}. The average gluconic acid production rate obtained in the shake flask experiments was within the range of 14 and 28 g l^{-1} d^{-1}. These results are corroborated with the findings of Ikeda et al. (2006). In the repeated batch cultivation with an air supply that was reported, the average gluconic acid production rate was 23.7 g l^{-1} d^{-1} in saccharified waste paper medium. These values also compared fairly well with the values available in the literatures (Sankpal and Kulkarni, 2002; Liu et al., 2003). The volumetric rate of substrate consumption (Q_s) in the media M1, M2 and M3 gave the average rate of 15 g $l^{-1}d^{-1}$, while media M3, M6 and M9 gave the average rate of 32 g $l^{-1}d^{-1}$. Ikeda et al. (2006) observed the average values of volumetric rate of substrate consumption (Q_s) from saccharified waste paper and glucose media as 11.0 g $l^{-1}d^{-1}$ and 29.3 g $l^{-1}d^{-1}$, respectively. These results compared well with this present study.

4. CONCLUSION

This study has shown that *Aspergillus niger* is capable of producing gluconic acid from breadfruit starch hydrolysate. The optimal media obtained consist of 100 g/l of breadfruit starch hydrolysate, urea as the nitrogen source and pH of 5.5 using 3 by 3 Graeco-Latin square experimental design method which produced 97.20 g/l gluconic acid. The initial pH of the media composition has significant effect on the production of gluconic acid and the pH of 5.5 was found to be optimum. The medium 5 has the highest biomass concentration of 45.6 g/l and the lowest concentration was 19.76 g/l of medium 1. This showed that L-glutamic acid gave highest yield of biomass concentration compared to ammonium sulphate.

REFERENCES

Adewusi, S.R.A., Udio, A.J. and Osuntogun, B.A., Studies on the carbohydrate content of breadfruit (*Artocarpus communis* Frost) from South Western Nigeria. Starch/Stärke 47: 287-294, 1995.

Table 2: Evaluation of Kinetic Parameters for Optimal Production of Gluconic Acid with Breadfruit Hydrolysate by Aspergillus niger under Shake Culture Fermentation Process

Media	P_t	S	Q_p	Q_s	Y'_{ps}	$\%Y_{acid}$	Y'_{px}	Q	μ	Y
1	44.72	0.09	14.47	15.82	0.857	79	2.43	0.020	0.00	0.314
2	96.40	0.25	25.18	27.60	0.960	88	2.240	0.022	0.010	0.380
3	100.23	1.09	25.10	32.50	0.827	76	2.055	0.029	0.014	0.317
4	46.26	0.10	15.19	15.31	0.929	85	2.400	0.007	0.003	0.359
5	96.40	0.23	25.06	27.86	0.957	89	2.009	0.020	0.010	0.420
6	91.75	1.75	23.06	32.23	0.764	70	1.803	0.025	0.014	0.334
7	46.26	0.10	15.41	14.64	0.949	87	2.452	0.015	0.006	0.370
8	97.20	0.18	25.01	27.41	0.960	90	2.166	0.024	0.011	0.375
9	108.71	1.38	27.58	31.25	0.898	82	2.292	0.023	0.010	0.336

Key: P_t – Final gluconic acid concentration (g/l); S – Residual sugar concentration (g/l); Q_p – Volumetric productivity ($g\ l^{-1}d^{-1}$); Q_s – Volumetric rate of substrate consumption; Y'_{ps} – Process product yield ($g\ g^{-1}$); Y'_{px} – Specific product yield ($g\ g^{-1}$); μ - Specific growth rate (h^{-1}); Q – Specific rate of product formation ($g\ g^{-1}d^{-1}$); Y'_{xs} – Growth yield coefficient ($g\ g^{-1}$)

Bao, J., Furumoto, K., Fukunaga, K. and Nakao, K., A Kinetic Study on Air Oxidation of Glucose Catalyzed by Immobilized Glucose Oxidase for Production of Calcium Gluconate. *Biochemical Engineering Journal*, 8: 91–102, 2001.

Betiku, E. and Ajala, E.O., Enzymatic Hydrolysis of Breadfruit Starch: Case Study with Utilisation for Gluconic Acid Production. *Ife Journal of Technology*, 19(1): 10-14, 2010.

Das, A. and Kundu, P.N., Microbial Production of Gluconic Acid. *Journal of Science and Industrial Research*, 46: 307–331, 1987.

Dirkx, J.M.H. and van der Baan, H.S., The Oxidation of Glucose with Platinum on Carbon as Catalyst. *Journal of Catalysis*, 67: 1–13, 1981.

Doran, P.M., Bioprocess Engineering Principles. *Academic Press, Harcourt Brace and Company, Publishers, London.* pp. 259-291, 1994.

Hustede, H., Haberstroh, H.J. and Schinzig, E., Gluconic Acid. In: Ullmann's Encyclopedia of Industrial Chemistry, *Weinheim, A* (12): 449-456, 1989.

Ikeda, Y., Park, E.Y. and Okuda, N., Bioconversion of Waste Office Paper to Gluconic Acid in a Turbine Blade Reactor by the Filamentous fungus *Aspergillus niger*, *Bioresource Technology*, 97: 1030–1035, 2006.

Laane, C., Pronk, W. Franssen, M. and Veeger, C., Use of Bioelectrochemical Cell for Synthesis of (Bio)chemicals. *Enzyme and Microbial Technology*, 6(4): 165-168, 1984.

Liu, J., Weng, L., Zhang, Q., Xu, H. and Ji, L., A Mathematical Model for Gluconic Acid Fermentation by *Aspergillus niger*. *Biochemical Engineering Journal*, 14: 137–141, 2003.

Magnuson, J. and Lasure, L, Organic Acid Production by filamentous fungi. In: Kacz, J. and Lange, L. (Eds.), Advances in Fungal

Röhr, M., Kubicek C.P. and Kominek, J., Gluconic acid. In: Rehm HJ, Reed G, editors. *Biotechnology* 6. USA: VCH Publishers, pp. 347-362, 1996.

Roukas, T., Citric Acid, Gluconic Acid Production from Fig by *Aspergillus niger* Using Solid-State Fermentation. *Journal of Industrial Microbiology Biotechnology*, 25: 298–304, 2000.

Sakurai, H., Lee, H.W., Sato, S., Mukataka, S. and Takahashi, J., Gluconic Acid Production at High Concentration by *Aspergillus niger* Immobilized on a Nonwoven Fabric. *J. Ferment. Bioeng.*, 67(6): 404-408, 1989.

Sankpal, N.V. and Kulkarni, B.D., Optimization of Fermentation Conditions for Gluconic Acid Production using *Aspergillus niger* Immobilized on Cellulose Microfibrils. *Process Biochemistry*, 37:1343-1350, 2002.

Shah, D.N. and Kotharis, R.M., Fermentative Production of Injectable-grade Calcium Gluconate, *Biotechnology Programme*, 7: 67–69, 1991.

Singh, O.V., Jain, R.K. and Singh, R.P., Gluconic Acid Production under Varying Fermentation Conditions by *Aspergillus niger*. *J Chem Tech Biotechnol*, 78: 208–212, 2003.

Biotechnology for Industry, Agriculture and Medicine, Kluwer Academic & Plenum Publishers, New York, pp. 307 – 340, 2004.

Miller, G.L., Use of Dinitro-salicyclic Acid Reagent for the Determination of Reducing Sugar. *Analytical Chemistry*, 31: 426-428, 1959.

Mukhopadhyay, R., Chatterjee, S., Chatterjee, B.P., Banerjee, P.C. and Guha, A.K., Production of gluconic acid from whey by free and immobilized *Aspergillus niger*, *International Dairy Journal*, 15: 299–303, 2005.

Olatunji, O. and Akinrele, A.I., Comparative Rheological Properties and Bread Qualities of Wheat Flour Diluted with Tropical Tuber and Breadfruit Flours. *Cereal Chem*, 55: 1-6, 1978.

Omobuwajo, T.O. and Wilcox, B.F., Microbes Associated with Field Spoilage of Breadfruit. *Lebensm.-Wiss.-u.-Technol.*, 22: 175-178, 1989.

Ramachandran, S., Fontanille, P., Pandey, A. and Larroche, C., Gluconic Acid: Properties, Applications and Microbial Production, *Journal of Food Technology, Biotechnology*, 44 (2): 185-195, 2006.

Rao, S. and Panda, T., Comparative analysis of calcium gluconate and sodium gluconate techniques for the production of gluconic acid by *Aspergillus niger*, *Bioprocess Engineering*, 8: 203–207, 1993.

Rao, S. and Panda, T., Critical analysis of the metal ions on gluconic acid production by *Aspergillus niger* using a treated Indian cane molasses, *Bioprocess. Engineering*, 10: 99–107, 1994.

Röhr, M., Kubicek C.P. and Kominek, J., Gluconic Acid. In: Rehm, H.J., Reed, G. (Eds.), Biotechnology, Vol. 3, Verlag Chemie, Weinheim, Germany, pp. 455–465, 1983.

Singh, O.V., Kapur, N. and Singh, R.P., Evaluation of agro-food by-products for gluconic acid fermentation by *Aspergillus niger* ORS-4Æ410. *World Journal of Microbiology & Biotechnology*, 21: 519–524, 2005.

Singh, O.V. and Singh, R.P., Bioconversion of Grape Must into Modulated Gluconic Acid Production by *Aspergillus niger* ORS-4.410, *Journal of Applied Microbiology*, 100: 1114-1122, 2006.

Solomon, B.O., Layokun, S.K., Idowu A.O. and Ilori, M.O. Prospects for the utilization of the endogenous enzymes in sorghum malt in the hydrolysis of starch: case study with utilization of breadfruit starch for ethanol production. *Food Biotechnology*, 8: 243 – 255, 1994.

Vassilev, N.B., Vassileva M.C. and Spassova, D.I., Production of gluconic acid by *Aspergillus niger* immobilized on a polyurethane foam, *Applied Microbiology & Biotechnology*, 39: 285–288, 1993.

Weir, C., *Fruit Tree Crop Production in the Caribbean Region* (Caribbean Development Bank, Barbados, 1982), 1982.

Znad, H., Markos, J. and Bales, V., Production of Gluconic Acid from Glucose by *Aspergillus niger*: Growth and Non growth Conditions, *Process Biochem.*, 39: 1341–1345, 2004.