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Glutamic Acid Production from Rice Husk Using *Corynebacterium glutamicum* Isolated from Soil

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Abstract: Many different biomass of agricultural origin holds remarkable potential for conversion into valuable products thereby presenting a double sharp edge importance of sustainable resource supply and environmental protection. Glutamic acid was produced from rice husk using a novel strain of *Corynebacterium glutamicum* and effects of parameters optimization such as substrate concentration, temperature, pH and inoculum size were determined during the fermentation process. The wild-type (Novel) strain was inoculated into 13 g/L of the pre-treated rice husk previously added to basal medium (pH 7.2), after which fermentation began. Fermentation broth from each flask was taken aseptically after 96 h and was assayed qualitatively and quantitatively. The acid-treated and alkali-treated rice husk gave the best glutamic acid yield of 10.40g/L and 9.08g/L respectively with the wild-type strain under predetermined optimum fermentation conditions. Out of the four parameters optimized, only substrate concentration was not found to be significant on the performance of the wild-type strain in glutamate production ($p > 0.05$). Acid-treated rice husk hydrolysate was found to be a better substrate for L-glutamate production by the wild-type strain of *C. glutamicum* under the optimum fermentation conditions determined.

Keywords: Rice Husk, Wild-Type, *C. glutamicum*, Optimization, Glutamate

1. Introduction

One of the most importantnon-essential amino acids is L-Glutamate. It is widely used in foods as a flavour enhancer. There is average annual production of about 1.8 million tons of monosodium glutamate via fermentative processes by bacteria of the coryneform group (Nakamura *et al*., 2006). There coryneforms are commonly found in the soil especially soils that are rich in organic matter. *Corynebacterium glutamicum* is catalase-producing and break down carbohydrates by fermentative metabolism (Blombach and Seibold, 2010). This organism is also auxotrophic to biotin but secretes L-glutamic acid in response to biotin limitation (Nottebrock *et al*., 2003). Several different substrates such as glucose, beet molasses and cassava residues are used for the production of glutamic acid. Other cheap agricultural residues such as rice husk and sugarcane bagasse could equally be used as alternative sources of carbon for the production of glutamic acid as reported by Vijayalakshmi and Sarvamangala (2011). Overall, optimum glutamic acid yield is obtained under optimum fermentation parameters irrespective of the strain type; whether wild or mutant forms of any organism capable of producing this particular amino acid.

2. Materials and Method

2.1. Collection of Samples

A total of four differentsoil locations at Samaru village were considered for sample collection to isolate *Corynebacteriumglutamicum*. A total of eleven soil samples were collected during the period of the study. Five (5) samples from different parts of flower bed around the Department of Microbiology, Ahmadu Bello University, Zaria, and two (2) samples each from paddock, chicken-run and sheep-pen withinSamaru village at a depth of about 10cm. Each sample was packaged in a clean polythene bag, labeled appropriately and then transported to the Department

of Microbiology, Ahmadu Bello University, Zaria for analyses.

About 1kg of fresh and fine-textured rice husk was also collected from rice milling station at Samaru village, SabonGari Local Government Area of Kaduna state. The rice husk sample was packaged into polythene bag, labeled appropriately and then transported to the Department of Microbiology, A.B.U. Zaria for analyses.

2.2. Treatment of the Rice Husk (Substrate Pre-treatment)

The method described by Rakesh *et al*. (2013) was adopted.

2.3. Alkaline Pre-treatment

A weighed amount of 25g of the fresh rice husk was placed in a 1000mL Erlenmeyer flask and 225 mL of 1.0M KOH solution was added. The flask was cotton- plugged and autoclaved at 121°C for 30 min. The material obtained after treatment was then filtered through muslin cloth and washed several times under running distilled water until no color was visible in the wash water and the pH adjusted to physiological value (7.2). The neutralized residue was then pressed manually to remove excess water and used for the enzymatic hydrolysis (Narasimha *et al*., 2011). A small portion of the treated biomass was dried in an oven at 70°C for 24 h and was ground to fine particle size in a laboratory mill for the proximate analysis (Rakesh *et al*., 2013) at the Department of Food Science, Institute of Agricultural Research, Ahmadu Bello University, Zaria.

2.4. Acidic Pre-treatment

The method described by Rakesh *et al*. (2013) was adopted with modifications. About 25g of the rice husk sample was added to a 1000mL flask and about 225mL of 1.0M sulphuric acid was added to the sample. The mixture was autoclaved at 121°C for 30 min and the material obtained after treatment was then filtered through muslin cloth and washed several times under running distilled water until no color is visible in the wash water and the pH adjusted to physiological value (7.2) following calcium hydroxide over-liming. The neutralized residue was then pressed manually to remove excess water and used for the enzymatic hydrolysis. A small portion of the treated biomass was dried in an oven at 70°C for 24 h and was ground to fine particle size in a laboratory mill for the proximate analysis (Rakesh *et al*., 2013) at the Department of Food Science, Institute of Agricultural Research, Ahmadu Bello University, Zaria.

2.5. Isolation and Characterization of Corynebacterium glutamicum

The following protocols were adopted during isolation and characterization of *Corynebacterium glutamicum;*

2.5.1. Media Preparation

The following media used were of analytical grade (OXOID) and were prepared according to manufacturer's instruction; Loeffler's Blood Serum Medium, Modified Hoyle's Medium, Sheep Blood Agar, Motility medium.

2.5.2. Isolation of Corynebacterium glutamicum

About 25g of each of the five soil samples was separately added to 225ml of sterile distilled water and a tenfold serial dilution was carried out to a final dilution of 10^{-5} using sterile normal saline. A loopful from each of the $1{:}10^2$ diluted soil suspensions was separately inoculated onto slants of Loeffler's medium by streaking. The inoculated slants were then incubated at 35°C for 48 hr. Discrete; well colonies were identified and sub-cultured on modified Hoyle's medium and incubated at 35°C for 48hr. The isolates were sub-cultured onto nutrient agar slants for subsequent identification and use.

2.5.3. Identification of Corynebacterium glutamicum

*Corynebacteriumglutamicum*isolates were primarily identified on the basis of the taxonomic properties such as morphology as well as cultural and biochemical properties (Bergey, 2004).

3. Preliminary Screening of the Isolates for Glutamic Acid Production

3.1. Screening Medium

The compositions of the screening medium used for L-glutamate production per 1000 ml of dH_2O is as follows; glucose, 5gm; calcium carbonate, 1gm; ammonium sulphate, 1gm; potassium di-hydrogen phosphate, 0.3gm; di-potassium hydrogen phosphate, 0.7gm; magnesium sulphate hepta-hydrate, 0.01gm; ferrous sulphate hepta-hydrate, 0.2mg; magnesium chloride tetra hydrate, 0.2mg; thymine hydro chloride, 20µg; and d-biotin, 10µg.

3.2. Culture Procedure

One hundred milliliters of screening medium was taken in each 250 ml flask, sterilized and inoculated with 24 hours old bacterial broth culture. Theflasks were incubated on a shaker incubator at 37°C for 96h at 180 rpm. Samples were taken after 96h and centrifuged at 5000rpm for 10 min. Supernatants were then examined for L- glutamic acid.

3.3. Qualitative Estimation of Glutamate

Qualitative analysis for L-Glutamate was done by paper chromatographic technique as described by Hassan *et al.* (2003).

3.4. Quantitative Estimation of Glutamate

About two milliliters of the supernatant from each of the fermented screening medium was taken separately in test tubes and two milliliters of 5% ninhydrin in acetone was added and heated for 15 min in boiling water bath. The tubes were then cooled to room temperature and glutamate was quantitatively estimated by taking readings at 570 nm using spectrophotometer with reference to the standard curve.

4. Glutamic Acid Production from Rice Husk by Submerged Fermentation

4.1. Basal Medium

For L-glutamic acid production by *C. glutamicum* through submerged fermentation, optimization of such parameters like substrate-water ratio, temperature, pH and inoculum size, cells were cultured in basal salt (BS) medium per litre. The basal salt medium comprised the following per litre: 5 g, $(NH_4)_2SO_4$; 5 g, urea; 2 g, KH_2PO_4; 2 g, K_2HPO_4; 0.25 g, $MgSO_4 \cdot 7H_2O$; 0.01 g, $FeSO_4 \cdot 7H_2O$; 0.01 g, $MnSO_4 \cdot 5H_2O$; 0.01 g, $CaCl_2 \cdot 2H_2O$; 0.03 mg, $ZnSO_4 \cdot 7H_2O$; 0.1 mg, H_3BO_4; 0.07 mg, $CoCl_2 \cdot 6H_2O$; 0.03 mg, $CuCl_2 \cdot 2H_2O$; 0.01 mg, $NiCl_2$; 0.1 mg of $NaMo_2O_4 \cdot 2H_2O$;200µg of biotin (pH 7.0). The initial pH was adjusted at 7.2 with 1N NaOH.

4.2. Submerged Fermentation

About 50ml of the basal medium was mixed separately with 13g each of the acid-pre-treated and alkali-pre-treated substrate (4:1 v/v) in 250ml Erlenmeyer flasks and labeled appropriately. Four (4) ml each of the 18hours-old culture of the wild-type were added appropriately and incubated at 37°C for 96hrs (Ahmed *et al*., 2013). The qualitative and quantitative analyses of the glutamic acid produced were carried out and results were recorded accordingly.

4.3. Parameters Optimized for L-glutamate Production

The following parameters were optimized; effects of substrate concentration of glutamic acid production, effect of pH on glutamic acid production, effects of inoculum size on glutamic acid production.

4.4. Analytical Methods

The glutamic acid produced was qualitatively and quantitatively detected with reference to standard curve.

4.5. Statistical Analysis

Data analysis was carried out through a statistical method using SPSS 13.0 software (Levesque, 2007). All the optimization parameters were analyzed by comparison of means through Paired t-test.

5. Results

5.1. Bacterial Strain Isolation, Identification and Screening for Glutamic Acid Production

Eight isolates were identified; seven of the isolates were confirmed to be *C. glutamicum* based on cultural, microscopic as well as biochemical characterizations as shown in Table 1.

The result of the screening for glutamic acid production by the isolates of *C. glutamicum* is shown in Table 2. The isolate from chicken-pen named as SFCD2 gave the maximum (0.25g/L) L-glutamic acid yield, with isolate from sheep-pen named as SFSD1 giving the least yield of 0.12g/L when determined spectrophotometrically at 570nm with reference to standard curve.

5.2. Determination of Optimum Conditions Affecting L-glutamic Acid Production by Wild- Type Strain of C. glutamicum

Effect of Substrates Concentration on Glutamic Acid Production

Various concentrations (1 to 5%) of each of the pre-treated substrates were investigated and it was found that 4% (w/v) of the acid-treated rice husk gave the highest production of glutamic acid (6.37 g/L) after 96 hours of incubation. The alkali-treated rice husk, gave the highest glutamic acid yield of 4.30 g/L at 4% of its concentration after 96 hours of incubation as shown in Figure 2.

Effect of Temperature on Glutamic Acid Production

Different temperatures (25, 30, 35 & 40°C) were used to determine the optimum temperature for L-glutamate production from wild *C. glutamicum* with 4% acid-treated and 4% alkali-treated rice husk using 5% inoculum. The maximum glutamate (10.1 g/L) was produced at 30°C. With increasing temperature, a decrease of glutamate yield was found as shown in Figure 3.

Effect of Inoculum Size on Glutamic Acid Production

Different inoculum concentrations (1, 3, 5, 7 and 9%) were used to find the optimum level for maximum glutamate production using wild *C. glutamicum* with pre-optimized conditions. Inoculum size of 7% produced the maximum glutamic acid (10.4 g/L). Thereafter, increasing the concentration of the inoculum resulted in a decreased glutamic acid yield as shown in Figure 4.

Effect of Initial pH on Glutamic Acid Production

The effects of various levels of initial pH (6.6, 6.8, 7.0, 7.2 & 7.4) were investigated by using 4% acid-treated, 4% of alkali-treated and 7% inoculum at 30°C with wild *C. glutamicum* as fermenting agent. Glutamic acid production of 16.06 g/L and 9.64g/L were observed for acid-treated and alkali-treated rice husk respectively at pH 7.0. Further decrease in glutamic acid yield was found with increasing pH as shown in Figure 5.

Table 1. *Cultural, Microscopic and Biochemical Characteristics of the Isolates.*

Isolate's		Growth on		Biochemical Tests								
Code	LM	MHM	GRM			Cat.	U	MR	VP	NR	GL	AH
HLS1	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisades	Mot. -	Haem γ	+	+	+	-	+	-	+

Isolate's		Growth on		Biochemical Tests								
Code	LM	MHM	GRM			Cat.	U	MR	VP	NR	GL	AH
HLS2	Whitish-yellow colonies	Black non-mucoid Colonies	Gram +ve cocci in clusters	-	β	+	-	+	-	+	+	±
SFCD1	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisade	+	γ	+	-	+	-	-	-	+
SFCD2	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisade	-	γ	+	±	+	-	+	-	+
SFSD1	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisade	-	γ	+	+	+	-	-	-	+
SFSD2	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisade	-	γ	+	+	+	-	-	-	+
SFHD1	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisade	-	γ	+	+	+	-	-	-	+
SFHD2	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisade	-	γ	+	+	+	-	-	-	+

Table 1. *Continue.*

Isolate's	Growth on			Sugar Fermentation							
Code	LM	MHM	GRM	Glc	Gal	Fru	Suc	Ara	Mal	Lac	Inference
				Mot. Haem							
HLS1	Whitish-yellow colonies	Black slightly mucoid colonies	Gram +ve Palisades	+	-	+	+	-	+	+	*C. glutamicum*
HLS2	Whitish-yellow colonies	Black non-mucoid Colonies	Gram +ve cocci in clusters	+	-	+	+	-	+	+	*Staphylococcus* spp.
SFCD1	Whitish-yellow Colonies	Black slightly mucoid colonies	Gram +ve Palisade	+	-	+	-	-	±	±	*C. glutamicum*
SFCD2	Whitish-yellow Colonies	Black slightly mucoid colonies	Gram +ve Palisade	-	-	-	±	+	-	-	*C. glutamicum*
SFSD1	Whitish-yellow Colonies	Black slightly mucoid colonies	Gram +ve Palisade	+G	+	+	+	+	+	+	*C. glutamicum*
SFSD2	Whitish-yellow Colonies	Black slightly mucoid colonies	Gram +ve Palisade	+G	+	+	+	+	+	-	*C. glutamicum*
SFHD1	Whitish-yellow Colonies	Black slightly mucoid colonies	Gram +ve Palisade	+G	+	+	-	-	-	±	*C. glutamicum*
SFHD2	Whitish-yellow Colonies	Black slightly mucoid colonies	Gram +ve Palisade	+G	+	-	±	-	-	±	*C. glutamicum*

KEY: LM; Loeffler's medium, MHM; Modified Hoyle's medium, GRM; Gram reaction and Morphology, Mot; Motility, Haem; Haemolysis, Cat; Catalase, U; Urease, MR; Methyl red, VP; Voges-Proskauer, NR; Nitrate Reduction, GL; Gelatin Liquefaction, AH; Aesculin Hydrolysis, Glc; Glucose, Gal; Galactose,Fruc; Fructose, Suc; Sucrose, Ara; Arabinose, Mal; Maltose, Lac; Lactose, G; Gas, +;Positive, -;Negative, ±; Weakly positive, β; Beta, γ; Gamma

Table 2. *Screening for L-Glutamic Acid Production by the C. glutamicum[a] Isolates.*

Isolate's Code	Glutamic Acid (g/L) b
HLS1	0.20
SFC D1	0.23
SFCD2c	0.25
SFSD1	0.12
SFSD2	0.21
SFHD1	0.19
SFHD2	0.23

KEY:[a]Shake flask fermentation at pH 7.0, Temperature: 37°C for 96h.
[b]Data are approximated to 2 decimal places

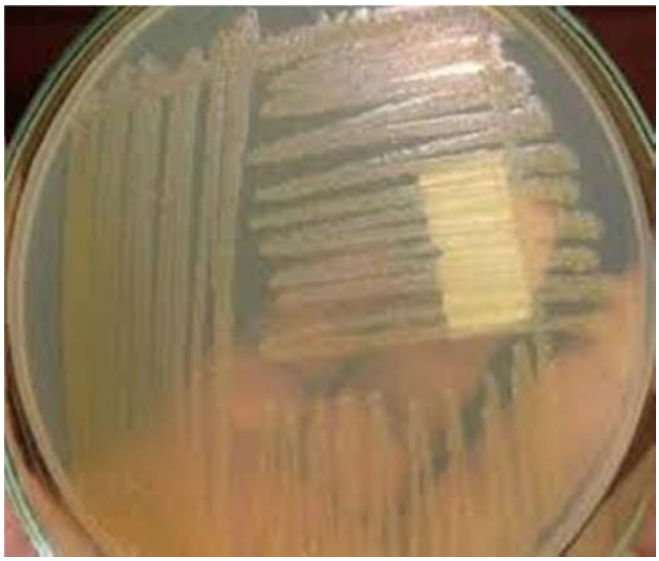
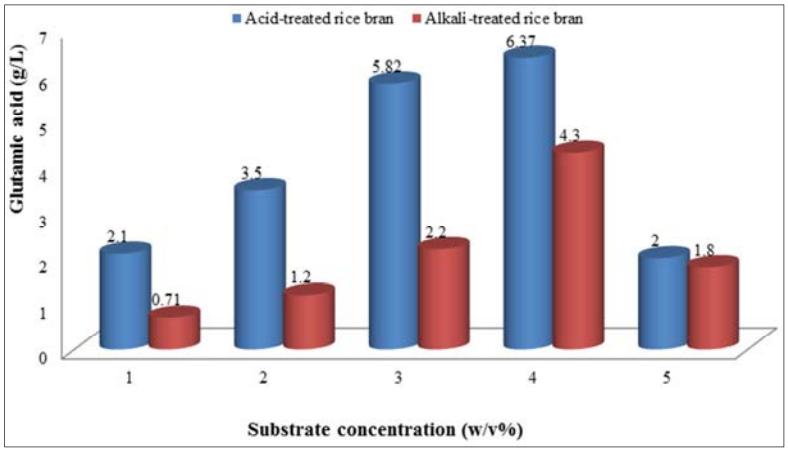
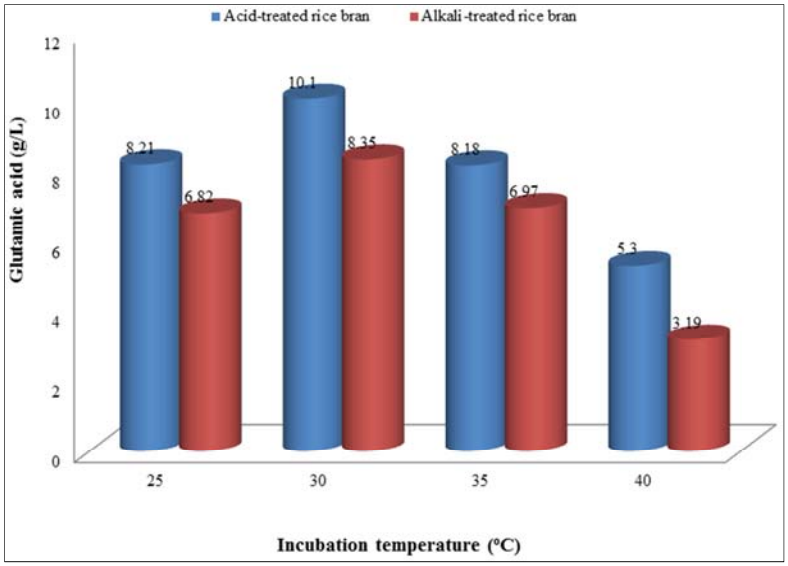


Figure 1. *Phenotypic appearance of the wild-type strain of C. glutamicumon modified nutrient agar medium.*



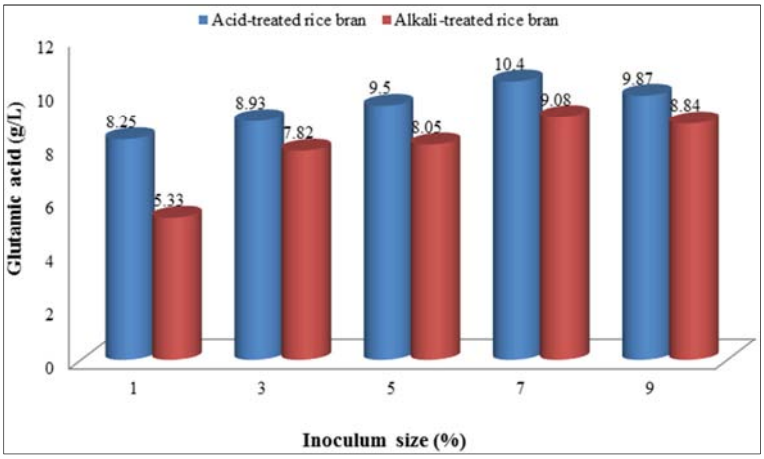
Acid-treated rice husk:p = 0.407, df = 4, t = -0.925; Alkali-treated rice husk:p = 0.183, df = 4, t = 1.607

Figure 2. *Influence of different substrates concentrations (w/v %) on glutamic acid production by wild-typeC. glutamicum.*



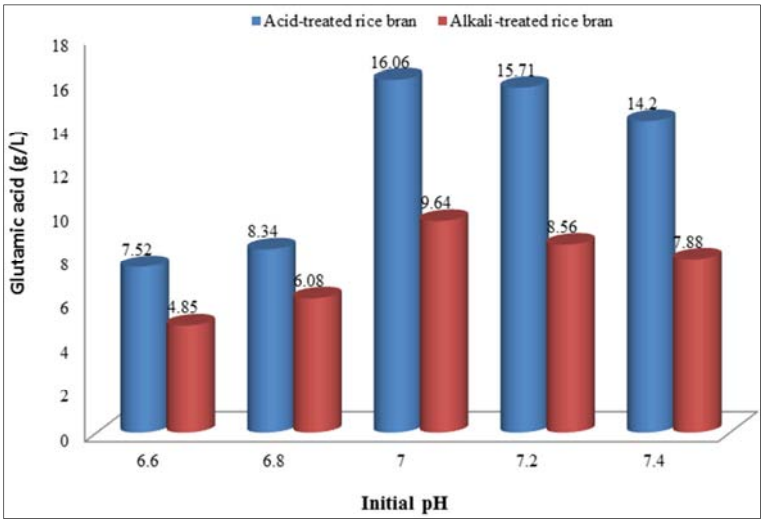
Acid-treated rice husk:p = 0.009, df = 3, t = 6.170; Alkali-treated rice husk: p = 0.008, df = 3, t = 6.395

Figure 3. *Effect of incubation temperature on glutamic acid production by wild-type C. glutamicum.*



Acid-treated rice husk:p = 0.016, df = 4, t= -4.013; Alkali-treated rice husk:p = 0.033, df = 4, t= -3.181

Figure 4. *Effect of inoculum size on glutamic acid production by wild-type C. glutamicum.*



Acid-treated rice husk:p = 0.036, df = 4,t = -3.098; Alkali-treated rice husk:p = 0.629, df = 4, t = -0.523

Figure 5. *Effect of initial pH on glutamic acid production by wild-type C. glutamicum.*

6. Discussion

In this study, a total of eleven (11) soil samples from four different locations were used for the isolation of *C. glutamicum*, of which a prevalence rate of (100%) was obtained with soil from paddock, chicken-pen and sheep-pen whereas soil from flower bed had the least prevalence of 20%. This might be due to the richness in the nutritional composition of the humic soil from the animal houses, whereas, the flower bed might have little organic matter as nutrient to the organism. This agrees with the findings of Zahoor *et al*. (2012) who reported a higher isolation rate (15.6%) from organically-rich soil than from nutrient poor soil with 5.74%. Out of the seven isolates confirmed to be *C. glutamicum* and screened for L-glutamic acid production, highest yield of glutamic acid of 0.25g/L was produced bythe isolate from chicken-pen (SFCD2) whereas,isolate named SFSD2 from sheep-penwas found to produce the lowest glutamic acid yield of 0.12g/L. This observed difference in glutamic acid yieldmight be due to the variability and adaptability in terms of the nutritional diversity of *C. glutamicum* as it is usually associated with the nature of the environment from which they were previously isolated. The highest glutamic acid yield obtained with SFCD2 in this study, is much lower than that obtained by Hadia *et al.*(2012) who reported a concentration of 1.5g/L after screening. This difference may be accounted for by the higher biotin concentration of 200µg used in this study as opposed to the lower concentration of 50µg used by Hadia *et al*. (2012).

After testing various concentrations of both acid-treated and alkali-treated hydrolysatesfor glutamic acid production bywild type strains of *C. glutamicum*in this study, 4% was found to be the optimum. The difference between the yield obtained at 4% and other concentrations with the wild-type from acid-treated hydrolysate (6.37g/L) and alkali treated hydrolysate (4.30g/L) was not statistically significant ($p>0.05$). These present findings are in agreement with the work of Chen *et al*. (2008).

The results of these present findings showed that the yields obtained with the wild-type from acid-treated hydrolysate

(10.10g/L) and alkali-treated hydrolysate (8.35g/L) at 30°C were all found to be significantly higher ($P< 0.05$) than those obtained at other temperatures. This is because cardinality of temperature plays a very important role in the growth and metabolism of an organism. Thus, growth and metabolic functions at extreme temperatures are greatly inhibited and sometimes become almost impossible. At low temperatures, enzymes get inactivated while membrane lipoproteins which are important in glutamate excretion become hardened. While, at extreme temperatures, enzymes and membrane proteins get denatured and therefore loss their activities. The results of present study are in line with the work of Sthiannopkao *et al*. (2001).

On the other hand,the yields obtained with the wild-type from acid-treated hydrolysate (16.06g/L) at pH 7.0 were all found to be significantly higher ($P< 0.05$) than those obtained at other pH, whereas, the yield obtained at the same pH of 7.0 with alkali-treated hydrolysate (9.64g/L) was not statistically significant ($P = 0.629$) as shown in Figure 4. The reason might be due to the physiological nature of the pH 7.0 at which best metabolic functions are carried out. Extreme pH of the fermentation media might have negatively affected the membrane stability (membrane fluidity), enzymatic activities as well as transport of nutrients for growth and metabolism, hence low yield of glutamate recorded.

Of all the inoculum sizes(1, 3, 5, 7 and 9%) used in this study, the trend of glutamic acid production under pre-determined optimum conditions showed that highestglutamic acid yield(10.40 g/L) was obtained from acid treated substrate than the alkali treated substrate (9.08g/L) by the wild-type strain with 7% of inoculum ($p < 0.05$). This might not be unconnected with the density-dependent bacterial communication (Quorum sensing) based on which metabolic functions of a microbial entity are determined.

7. Conclusion

The use of rice husk as a locally available and nutrient-rich raw material when treated with acid could enhance glutamic acid production by the wild-type strains of *C. glutamicum*, and this would be of economic and environmental benefits through the establishment of cost-effective local glutamic acid industry in Nigeria as well as cleaning the environment of agricultural wastes.

References

[1] Ahmed, Y. M., Khan, J. A., Abulnaja, K. A. andAl-Maliki, A. L. (2013). Production of glutamic acid by *Corynebacteriumglutamicum* using dates syrup as carbon source. *African Journal of Microbiology Research*, 7(19): 2072.

[2] Amin, G. A. and Al-Talhi, A. (2007). Production of L-glutamic Acid by Immobilized Cell Reactor of the Bacterium *Corynebacteriumglutamicum*Entrapped into Carrageenan Gel Beads. *World Applied Science*, *Journal*, 2: 62-67.

[3] Bergey's Manual of Determinative Bacteriology. (2004). Eds., John G. Holt *et al.,* 9thedn. The Williams and Wilkins, Baltimore, p.565.

[4] Blombach, B. and Seibold, G. M. (2010) Carbohydrate metabolism in *Corynebacteriumglutamicum* and applications for the metabolicengineering of L-lysine production strains. *Applied Microbiology and Biotechnology*, 86(5): 1313-1322.

[5] Chen, X., Chen, S., Sun, M. and Yu, Z. (2008). High yield of glutamic acid production from *Bacillus substilis* by solid state fermentationusing swine manure as the basis as solid substrate. *Bioresource Technology*, 96: 1875-1879.

[6] Hadia, G., Shah, A. and Younis, N. (2012). Fermentative Production of Glutamate by Newly Isolated Soil Bacteria. *International Journal of Pharmaceutical & Biological Archives*, 3(6): 1368-1376.

[7] Hassan, B., Asghar, M., Nadeem, S., Zubair, H., Muzammil, H. M. and Shahid, M. (2003). Isolation and Screening of Amino acids-Producing Bacteria from Milk. *Biotechnology,* 2(1): 18-29.

[8] Jyothi, A. N., Sasikiran, K., Nambisan, B. and Balagopalan, C. (2005). Optimization of glutamic acid production from cassava starch factory residues using *Brevibacteriumdivaricatum. Processes in Biochemistry*, 40(11): 3576-3579.

[9] Levesque, R. (2007). SPSS Programming and Data Mangement. AGiude for SPSS and SAS Users, fourth edition, SPSS Inc., Chicago III.ISBN 1- 56827-390-8.

[10] Nakamura, J., Hirano, S. and Ito, H. (2006). L-Glutamic Acid Producing Microorganism and a Method for Producing L-Glutamic Acid. U.S. patent US20060141588A1.

[11] Nottebrock, D., Meyer, U., Krämer, R. and Morbach, S.(2003). Molecular and biochemical characterization of mechanosensitive channels in *Corynebacteriumglutamicum.* FEMS *Microbiology Letters*, 218: 305-309.

[12] Rakesh, K. T. and Devendra, P. S. (2013). Acid and Alkaline Pre-treatment of Lignocellulosic Biomass to Produce Ethanol as Biofuel. *International Journal of Chemical Technology Research*, U.S.A., 5(2): 729.

[13] Sthiannopkao, S., Danner, H. and Braun, R. (2001). Use of Grass Sap as an Ingredient in Glutamate Production. *Thammasat International Journal of Science and Technology*, 6: 3-4.

[14] Vijayalakshm, P. and Sarvamangala D. (2011). Production of L-glutamic acid by *Arthrobacterglobiformis* MTCC 4299 fruits of Mimusops Elengilinn. *International Journal of Applied Biology and Pharmaceutical Technology*, 2: 167-173.