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Full Lenght Research.

Production of L-glutamic acid from *Bacillus* isolates cultivated on agro-industrial wastes containing medium.

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An investigation was carried out to test the potential of using agro-industrial wastes as fermentation medium for the production of L-glutamic acid.The bacteria strains used for the study included *Bacillus subtilis* (UG1) from "Ugba", *Bacillus licheniformis* (OG1) from "Ogiri", *B.pumilus* (DD4) from "Dawadawa" and *B.polymyxa* (OG7) from "Ugba" and reference strain *Corynebacterium glutamicum* ATCC 13032.The agro-industrial wastes tested were hydrolysates of corn pomace and cassava peel.The production of L-glutamic acid was done under the single(when pineapple wastes) and double(starch based)stage fermentations.The study showed that pineapple was the best substrate for L-glutamic acid production:i.e the highest production of 6.2mg/ml was for *B.licheniformis* OG4 while the lowest production of 5.0mg/ml was from *B.pumilus* DD4.Corn pomace was the least efficient in enhancing L-glutamate production giving 4.9mg/ml from *B.licheniformis* as the highest and 4.2mg/ml from *B.subtilis* as the lowest.The rapid consumption of reducing sugar in the pineapple waste medium brought about a drastic fall in the pH to 4.0 at 72h of fermentation and an increase in L-glutamic acid production. Utilization of the agro-industrial wastes for L-glutamic acid will be of immense benefit at preventing the pollutional hazards associated with these wastes.

Keywords: L-glutamic acid, agro-industrial wastes, fermented vegetable proteins, fermentation.

Introduction

In recent years, bioconversion and utilization of agro-industrial wastes into useful products has been receiving attention both in the developed and developing countries of the world. This is because majority of these waste contain substantial amount of biodegradable matter, which when allowed decomposing produce noxious gases, which pollute the environment thereby compounding the problem of environmental sanitation. The management (including disposal) of agro-industrial waste is also often expensive. More over, all these wastes and residues are potential sources for the generation of new wealth, particularly in developing countries, if the appropriate technologies for their bioconversion can be developed. Some of the agro-industrial wastes are potentially useful in compounding industrial media where they can serve as cheap sources of nutrients for microbial growth and biosynthesis of useful products such as amino acids (Okafor, 1977).Monosodium glutamate is the highest produced amino acid.It is used in huge quantities as flavour enhancers throughout the world and its current production is 350,000 tonnes per year(Kawakita and Saeki,1986).

In the United States alone, it was reported that the market value for MSG was 55 million dollars. In Japan, current price for MSG in international Trade is at an average of 2 dollars per kg. The international price of MSG is at 1,500-1,800 dollars per tonne. In 1978, and 1988, about 20 and 25 million naira was spent respectively by Nigeria food industries in the importation of food seasonings.

Nigeria is the largest producer of cassava in the world with an annual output of over 34 million tons (Anon, 1994). However, massive utilization of cassava as staple food in the country limits its use in glutamate fermentation. In the alternative, cassava peel, corn waste which contains 5-7% starch could be provided as cheaper and readily available carbon source for potential glutamate fermentation. Pineapple peel is also available in large quantities as waste. The use of wastes will help to reduce the pollution that results from their indiscriminate disposal. To investigate the use of corn, cassava peel and pineapple waste as substrate for L-glutamic acid is the focus of this paper.

Materials And Methods

PROXIMATE ANALYSIS OF AGRO INDUSTRIAL WASTES.

Moisture Content Determination

The moisture content of the Agro-industrial wastes of cassava peel, corn pomace and pineapple waste were determined. Five grams of each sample were weighed in duplicates into pre-weighed aluminium drying dishes. The samples were dried to constant weight in at 105^0C for 4h (AOAC, 1990).

$$\%\text{Moisture Content} = \frac{M_1 - M_2}{M_1 - M_0}$$

M_0 = weight of Aluminium dish
M_1 = weight of fresh sample plus dish.
M_2 = weight of dried sample plus dish.

Crude Protein Determination

For this Kjedahl nitrogen method was used. One gram of the dried sample was introduced into the digestion flask. Kjedahl catalyst (5 selenium tablets) was added to the samples. Twenty millilitres of concentrated acid were added to ash sample and fixed to the digester for 8h until a clear solution was obtained. The cooled digest was transferred into a 100ml volumetric flask and made up to mark with distilled water. The distillation apparatus was set and rinsed for 10mins after boiling. Twenty millilitres of boric acid were pippetted each conical flask with 5 drops of methyl red indicator and diluted with 75ml distilled water. Ten millilitres of digest were made alkaline with 20ml of NaOH (20%) and distilled. The steam exit of the distillator was closed and the change of colour of boric acid solution to green was timed. The mixture was distilled for 15minutes (AOAC, 1990). The filtrate was later titrated against 0.1NHCl. The percentage total nitrogen was calculated as follows:

$$\% \text{ Total Nitrogen} = \frac{\text{Titre Value} \times \text{Normality} \times 0.014 \times 100}{\text{Sample weight}}$$

N =Normality of Acid.

% Crude Protein = %total nitrogen × 6.25

6.25 = Conversion factor

Crude Fibre Determination

The trichloro acetic acid method of Joslyn (1970) , was used. The samples were defatted with petroleum ether. Five grams of defatted sample were weighed into 600ml beakers and 100ml trichloroacetic acid (TCA) was added. The samples were boiled and refluxed for 4minutes. The cooled samples were filtered with filter paper whatman no. 4. The residues were washed six times with hot distilled water and once with methylated spirit. The samples were transferred on to porcelain crucibles and dried in an oven overnight at 100^0C. The samples were cooled in a desiccators, weighed, then ashed in a muffle furnace at 600^0C for 6h and weighed again after cooling. The loss in weight during incineration was equivalent to the amount of crude fibre.

$$\% \text{ Crude fibre} = \frac{(\text{weight A}) - (\text{weight B}) \times 100}{\text{Sample weight}}$$

Weight A = Weight of sample after drying.

Weight B = weight of sample after ashing.

Crude Fat Determination

The samples were dried and milled properly. Five grams of samples were weighed and put in thimble and plugged and cotton wool. The thimbles were dried and inserted into a soxtec system HT2. The extraction cups were dried, weighed and 25ml petroleum ether were added to each cup. The cups were inserted into the soxtec and the samples extracted for 15minutes in "boiling position and 35minutes in rinsing" position (AOAC, 1990). The percentage fat in the sample was calculated as follows:

$$\% \text{ Fat} = \frac{(W_1 - W_2)}{W_1} X \frac{100}{1}$$

W_1 = Weight of the sample

W_2 = Weight of the empty cup

W_3 = Weight of the cup with extracted oil

Ash Determination

Samples were collected and dried to constant weight. Five grams of dried samples weighed in porcelain crucible ignited and weighed.

Organic matter was charred by igniting the material on the hot plate in the fume cupboard. the crucibles were placed in the muffle furnaces and maintained at 600^0C for 6hr. They were cooled in the desiccators and weighed immediately.(AOAC, 1990).

$$\% \text{ Ash} = \frac{(\text{weight of crucible + Ash}) - (\text{weight of crucible}) \times 100}{\text{Sample weight.}}$$

Carbohydrate Determination

This was obtained by subtracting from 100, the sum addition of the percentage moisture, ash, protein, fat, and crude fibre. The remaining value is the carbohydrate content in the sample.

Isolation of *Bacillus spp.*

Isolation method for *Bacillus spp* from vegetable proteins of Iru, Ugba and Dawadawa was carried out as reported by Lawal, (2005).

SCREENING AGRO INDUSTRIAL WASTES AS SUBSTRATES FOR L-GLUTAMIC ACID PRODUCTION.

Preparation of agro-industrial waste samples

Cassava peel and corn pomace waste sample were obtained from the pilot sections of the Federal Institute of Industrial Research Oshodi, Lagos State Nigeria. Pineapple wastes are collected in large quantities from a local market at Mushin, Lagos Nigeria. The different waste samples were dried in a cabinet tray drier (Mamert vertical type) at 60^0C for 5days in the order to concentrate, the starch (about 45%) and sugar (42%) for starch and sugar based substrates respectively. The dried samples are later milled using Hammer mill (scotmec model) and sieved to obtain a fine flour (0.2µm), which was packaged and stored in the refrigerator until needed.

The agro-industrial wastes of cassava peel and corn pomace used were first hydrolysed using amylase enzymes produced as follow:

Amylase enzyme production

A culture of amylase producing fungus *Aspergillus niger* ATCC 16404 was obtained from the culture collection unit Division of Biotechnology Division, Federal Institute of Industrial Research Oshodi (FIIRO) Lagos Nigeria. It was maintained on potato dextrose agar (PDA) slant at 4^0C and subcultured bimonthly on the same medium.

Rice bran obtained from a local farm in Agege, Lagos, Nigeria was used as substrate. The production was carried out as described by Akpan *et al.,(*1995). Aqueous extract of rice bran was prepared by autoclaving rice bran suspension in (2%w/v) at 20^0C for 15minutes. The autoclaved suspension was passed

through cheese cloth to remove solids. Potassium phosphate (KH_2PO_4 0.1%w/v), Magnesium sulphate (MgSO4. 7H2O, 0.5 %(w/v), glucose 2% (w/v) were added to the clarified rice bran to form the basal medium.

The rice basal medium was dispensed as 100mls into 250-ml Erlenmeyer flasks and sterilized at 120^0C for 15minutes. The autoclaved medium was later inoculated with 0.5% (w/v) spore suspension of 48h old culture of *Aspergillus niger* ATCC 16404and incubated at 35^0C on a rotary shaker at 300 rpm for 5days. The enzymes produced were centrifuged from the culture broth at 5^0C and 10,000 r.p.m for 30 minutes. The enzyme was precipitated from the centrifuged broth by ammonium sulphate and there after assayed using 0.5ml of 4% soluble starch in 0.1m acetate buffer (pH 4.0) at 55^0C for 1 h.(Akpan *et al.,* 1995). Reducing sugar concentration released was determined quantitatively using the method of Miller (1959) and reference made to a standard curve for glucose .

Hydrolysis of agro-industrial wastes

Cassava peel and corn pomace prepared were both hydrolysed using the produced amylase enzyme following the methods of Carr *et al.,(*1982) and Anyakorah *et al.,(*1998)as follows.

The powdered cassava peel and corn pomace wastes samples earlier prepared were separately weighed (30%w/v) into 500ml beakers and gelatinized on hot plate magnetic stirrer. The gelatinized samples were later saccharified using reaction mixture made up of one part of appropriately diluted enzyme and four part of the gelatinized waste samples and incubated at 90^0C for 1h.

EVALUTION OF HYDROLYSATES OF CASSAVA PEEL, CORN POMACE AND PINEAPPLE WASTES FOR L-GLUTAMIC ACID PRODUCTION.

Pineapple, hydrolysates of corn and cassava wastes (fermentable sugars, 5.0g), were separately added to KH_2PO_4, 1.0g;$MgS0_4$, $7H_2O$, 0.5g;$FeSO_4.7H_2O$, 0.05g; biotin, 3ug; distilled water, 100mls.(Ph 7.2). The media were separately sterilized in an autoclave at 115^0C for 10 minutes and later inoculated in separate flasks with 1ml (2.6×10^6 cfu/) of the test isolates. Incubation was at 32^0C on a rotary shaker at 180rpm for 72h. (Gutcho, 1973). During the production process for L-glutamic acid, the following were determined

Cell growth in production medium

Cell growth was measured turbidometrically at 540nm using a spectrophotometer (Miltroy spectronic 20D model) against a blank made with distilled water.

pH determination

The pH of the fermenting medium was determined with the aid of a previously standardized pH meter (Unicam 9450 model). The pH meter was calibrated using PH 4.0 and Ph 7.0 buffers.

Determination of Reducing Sugar

Reducing sugar content was determined by the method of Miller (1959) as follows; 1ml of the sugar extract was added to 2ml of 3,5, DNSA reagent prepared by adding 60g of potassium sodium tartarate to 30g of sodium hydroxide and 1gm of DNSA in 200mls of distilled water. The mixture was heated in boiling water for 5 minutes and then cooled under running tap water.

The absorbance of the resulting coloured solution was read using the spectrophotometer (spectronic 20D model) at 540nm against a blank made by substituting the sugar extract with distilled water. The reducing sugar contents were determined by reference to a standard curve of known concentration for glucose.

Determination of L-glutamic acid

This was determined qualitatively and quantitatively following the method of spies (1957) was used.It was estimated by ninhydrin colour reaction and measuring the absorbance at 570nm.From the clarified sample of each aliquot,of the fermented medium,1ml was taken and added to 1ml of freshly prepared ninhydrin reagent.The mixture was heated in water bath for 5minutes and then cooled under a running tap.The absorbance of the resulting coloured solution was read using a spectrophotometer(Miltroy spectronic 20D model) at 570nm against a blank made by substituting the extract with distilled water.The glutamic acid content in the sample was determined by reference to standard curve of known concentration of L-glutamic acid.

Result

Proximate analysis of agro-industrial wastes

Prior to the use the of agro-industrial wastes for growth and glutamic acid production by the micro-organism, their proximate analyses were determined. The result (Table 1) showed that pineapple waste had the highest moisture content of 71.9% while cassava peel had the least with 10.8%. The protein content was height in cassava peel with 3.06% and least with corn pomace 2.8%. Crude fibre was highest in pineapple waste with 7.1% and least with cassava peel 5.2%. The fat content (only on dry basis) had cassava peel being highest with 5.42% and least with pineapple waste 3.2% and least with corn pomace 2.6%. The carbohydrate content was highest in corn pomace 71.6% and least in pineapple waste with 12.4%. The high level of carbohydrate in cassava and corn pomace gave an indication that they could be hydrolysed to fermentable sugars. (Table 1)

Table 1:Proximate analysis of waste samples.

Sample	Moisture (%)	Proteins (%)	Crude fibre (%)	Fat (% dry matter)	Ash (%)	carbohydrate (By difference)
Cassava peel	10.8±0.05	3.06±0.01	5.2±0.01	5.420.01	3.2±0.01	67.78±0.02

Table 1 continues.

Corn pomace	12.4±0.03	2.81±0.02	4±0.02	4.20±0.01	2.6±0.01	71.6±0.05
Pineapple waste	71.9±0.02	3.2±0.01	7.1±0.05	2.30±0.02	3.1±0.01	12.4±0.05

Values represent the means (n=3) ± S.D.

Changes in the pH of agro-industrial wastes with fermentation period.

Changes in the pH during fermentation of hydrolysed agro-industrial wastes by all the test isolates and reference strain were studied. A pH range of 3.8-4.7 was observed after fermentation of corn pomace hydrolyses by the test isolates and the reference strain (Tables 2, 3, 4, 5, and 6). For cassava peel hydrolysates the pH range was 3.8-4.8 and for pineapple waste it was 3.8-6.0 (Tables 2, 3, 4, 5, and 6).

Changes in the pH of the fermenting waste samples by *B.subtilis* UG1 showed that corn pomace, cassava peel hydrolysates and pineapple wastes had pH value of 4.5, 4.8 and 5.0 respectively from an initial pH value of 7.5 (Table 2).

Fermenting waste samples by *B.pumilus* DD4, had pH values 4.3, 4.0 and 4.41 for corn pomace, cassava peel hydrolysates and pineapple wastes respectively from an initial pH value of 7.5 (Table 3).

B. licheniformis OG4, had pH value change from an initial value of 7.5 to 4.7, 4.0 and 4.1 respectively for corn pomace, cassava peel hydrolysates and pineapple waste respectively (Table 4).

B.polymyxa OG7, had pH change from an initial pH value of 7.5 to 4.0, 4.0 and 3.8 for corn pomace, cassava peel hydrolysates and pineapple respectively during fermentation.(Table 5).

Changes in pH waste sample of corn pomace, cassava peel hydrolysates and pineapple waste fermented with *C. glutamicum* ATCC 13032 had its pH value of 7.5 which dropped to 3.8, 3.8 and 4.2 respectively (Table 6).

Table 2: Changes in pH during fermentation of waste samples by *Bacillus. Subtilis* UGI.

Time(h)	Waste samples		
	Corn pomace hydrolysate	cassava peel hydrolysates	pineapple waste
0	7.5±0.00	7.5±0.00	7.5±0.00
24	5.8±0.02	5.5±0.03	6.3±0.01
48	4.9±0.02	5.3±0.01	6.0±0.02
72	4.6±0.05	5.0±0.02	5.4±0.03
96	4.5±0.02	4.8±0.01	5.0±0.05

Values represent the means (n=3) ± S.D

Table 3: Changes in pH during fermentation of waste samples by *Bacillus.pumilus DD4*.

Time(h)	Waste samples		
	Corn pomace hydrolysate	cassava peel hydrolysates	pineapple waste
0	7.5±0.00	7.5±0.00	7.5±0.00
24	5.2±0.02	5.4±0.03	5.8±0.01
48	4.7±0.05	5.0±0.01	5.2±0.02
72	4.3±0.03	4.9±0.02	4.8±0.04
96	4.0±0.02	4.3±0.02	4.41±0.05

Values represent the means (n=3) ± S.D

Table4: Changes in pH during fermentation of waste samples by *Bacillus.licheniformis* OG4 .

Time(h)	Waste samples		
Corn pomace hydrolysate		cassava peel hydrolysates	pineapple waste
0	7.5±0.00	7.5±0.00	7.5±0.00
24	5.2±0.02	4.8±0.05	5.8±0.02
48	4.9±0.05	4.6±0.01	4.6±0.02
72	4.6±0.02	4.4±0.02	4.3±0.03
96	4.7±0.05	4.0±0.01	4.01±0.03

Values represent the means (n=3) ± S.D

Table 5: Changes in pH during fermentation of waste samples by *Bacillus.polymyxa* OG7.

Time(h)	Waste samples		
	Corn pomace hydrolysate	cassava peel hydrolysates	pineapple waste
0	7.5±0.00	7.5±0.00	7.5±0.00
24	5.6±0.02	5.4±0.03	5.0±0.02
48	5.0±0.02	5.1±0.02	4.3±0.02
72	4.7±0.01	4.3±0.02	4.0±0.02
96	4.0±0.02	4.0±0.01	3.8±0.01

Values represent the means (n=3) ± S.D

Table 6: Changes in pH during fermentation of waste samples by *Corynebacterium glutamicum* ATCC 13032.

Time(h)	Waste samples		
	Corn pomace hydrolysate	cassava peel hydrolysates	pineapple waste
0	7.5±0.00	7.5±0.00	7.5±0.00
24	4.3±0.05	4.8±0.01	5.4±0.05
48	4.1±0.02	4.3±0.02	5.1±0.01
72	4.0±0.03	4.0±0.03	4.3±0.02
96	3.8±0.02	3.8±0.05	4.2±0.02

Values represent the means (n=3) ± S.D

Growth of test isolates on the agro-industrial wastes during fermentation.

Studies on the growth of *B. subtilis* UG1, *B. pumilus* DD4, *B. licheniformis* OG4, *B. polymyxa* OG7, *C. glutamicum* ATCC 13032 on the fermenting hydrolysates of cassava, corn pomace and pineapple wastes are presented in Figures 1, 2, 3, 4 and 5. Pineapple wastes supported the best growth by all the isolates with an optical density range of 0.22- 1.82 and corn pomace hydrolysates least utilized by the isolates with an optical density range of 0.22- 0.93. Hydrolysates of cassava peel on the other hand, highest optical density of 0.90 was observed in *B. polymyxa* OG7 and *B. pumilus* DD4; and least in *B. licheniformis* OG4 and *C. glutamicum* ATCC 13032 with optical density of 0.80.

Increased growth by the test isolates and the reference strain in hydrolysates of corn, cassava and pineapple wastes was observed up to 72 hours followed by decline, Figures 1, 2, 3, 4 and 5

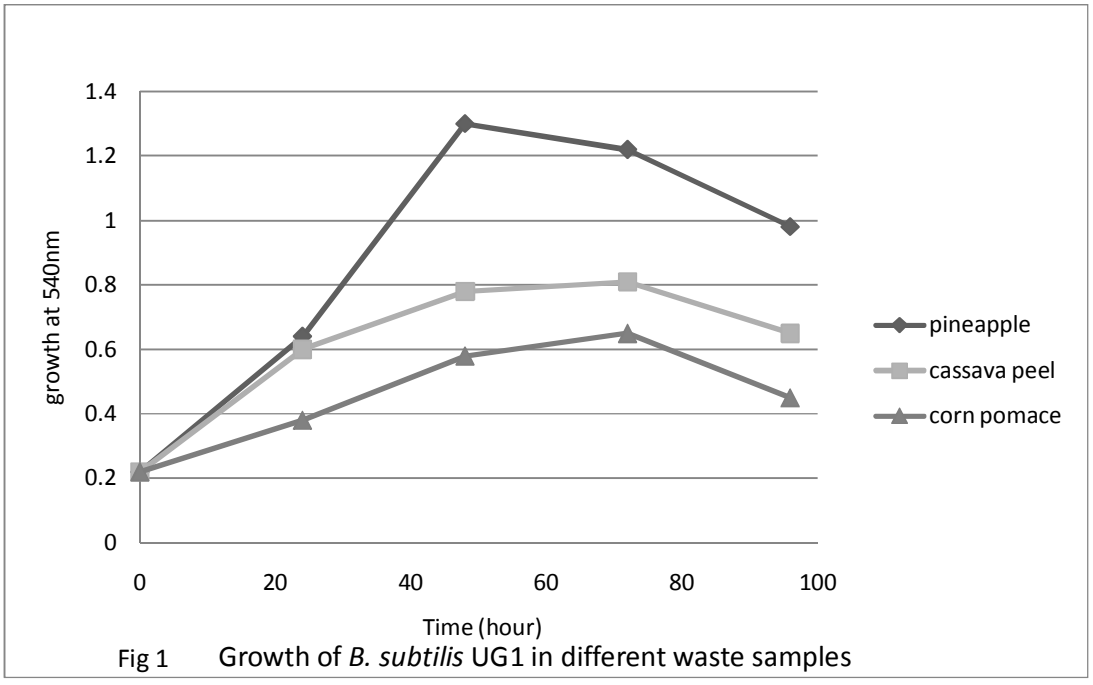

Fig 1 Growth of *B. subtilis* UG1 in different waste samples

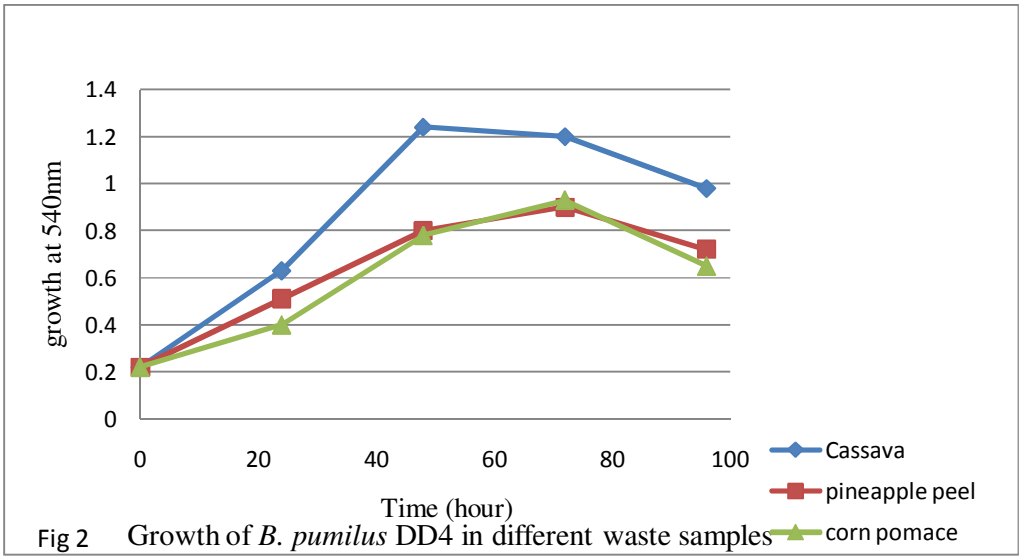

Fig 2 Growth of *B. pumilus* DD4 in different waste samples

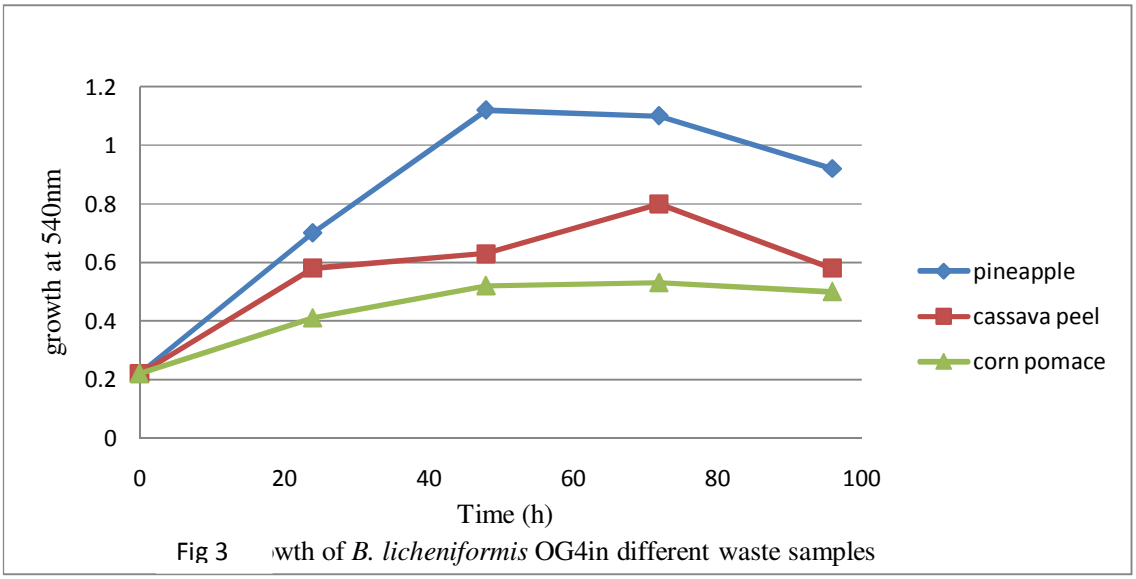

Fig 3 wth of *B. licheniformis* OG4in different waste samples

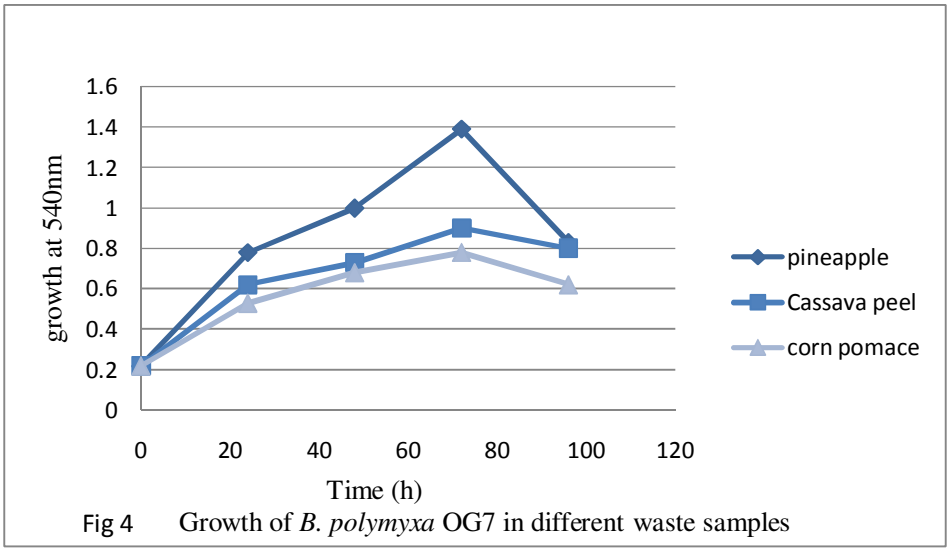

Fig 4 Growth of *B. polymyxa* OG7 in different waste samples

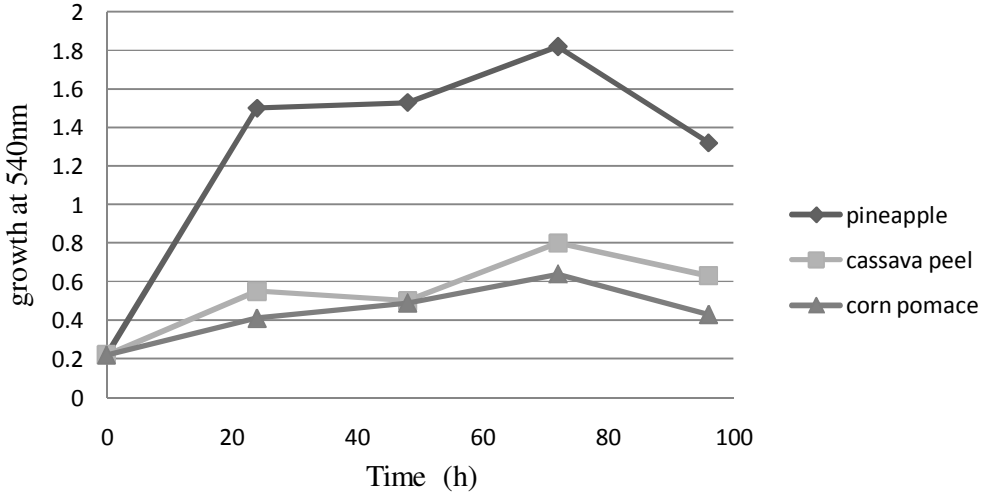

Fig 5 Growth of *C. glutanicum* ATCC 13032 in different waste samples

Changes in glucose and L-glutamic acid in waste samples.

The result of the quantity of glucose consumed and the amount of L-glutamic acid produced by the test isolates and the reference strain using pineapple wastes were as shown in Figures 6, 7, 8, 9 and 10. The test isolates were to utilize the glucose in pineapple wastes for the production of glutamic acid. *C. glutamicum* ATCC 13032 the highest glutamic acid (7.20mg/ml) while the level of glucose dropped to less that 15% *B. pumilus* DD4, produced the least glutamic acid (5.8mg/ml) while the level of glucose dropped to 37.5%.

Changes in the level of glucose and the amount of glutamic acid produced by the test isolates and reference organism in corn pomace hydrolysates is presented in Figures 11, 12, 13, 14 and 15. The result showed that *B. licheniformis* OG4, produced the highest glutamic acid (5..0mg/ml) while the level of glucose dropped to 29.28%. *B. subtilis* UGI, produced the least glutamic acid (4.10mg/ml) while the level of glucose dropped to 28.5%.

Utilization of glucose in cassava peel hydrolysed for production of glutamic acid revealed that *B. polymyxa* OG7, produced the highest glutamic acid (5.32mg/ml) while the glucose level dropped to 29.06%. *C. glutamicum* ATCC 13032 produced 5.4 mg/ml of glutamic acid while the level glucose dropped to 34.5%. Figures 16, 17, 18, and 19.

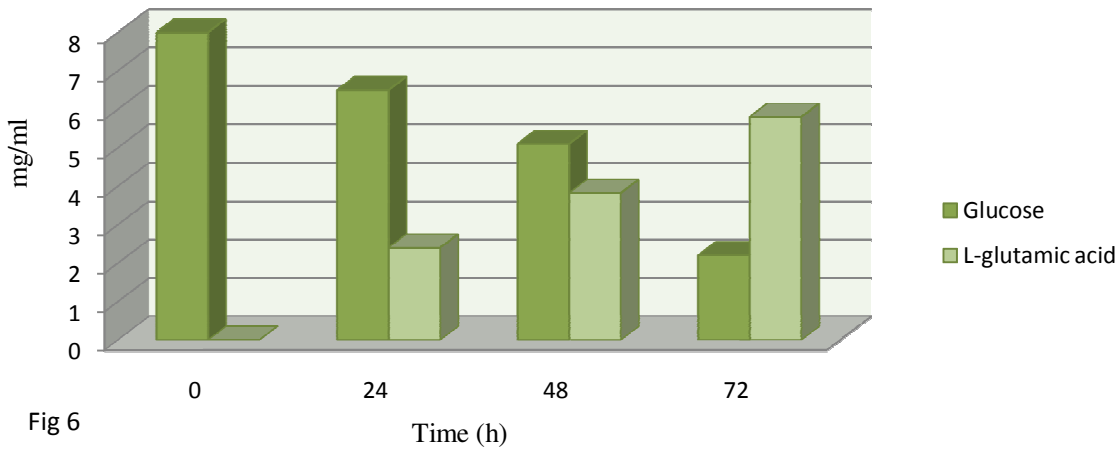

Fig 6 Changes in glucose and L-glutamic acid in pineapple waste by *B. pumilus* DD4

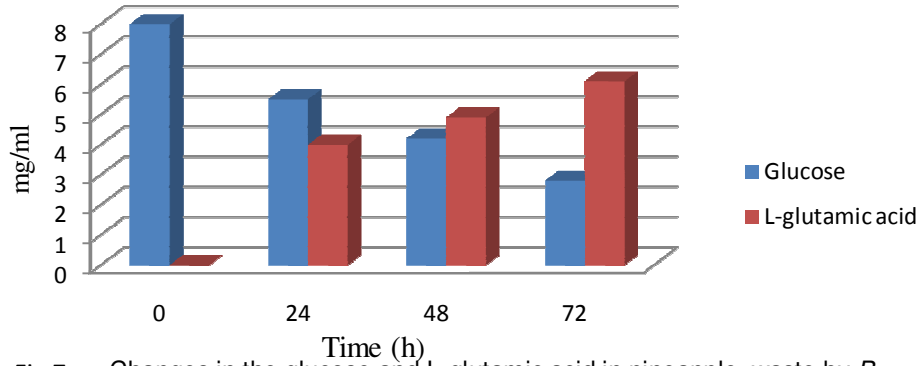

Fig 7 Changes in the glucose and L-glutamic acid in pineapple waste by *B. licheniformis* OG4

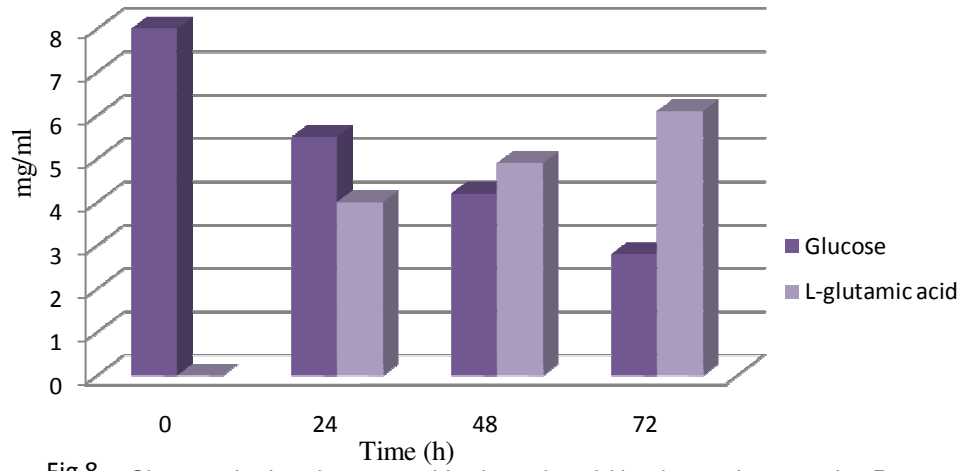

Fig 8 Changes in the glucose and L-glutamic acid in pineapple waste by *B. polymyxa* OG7

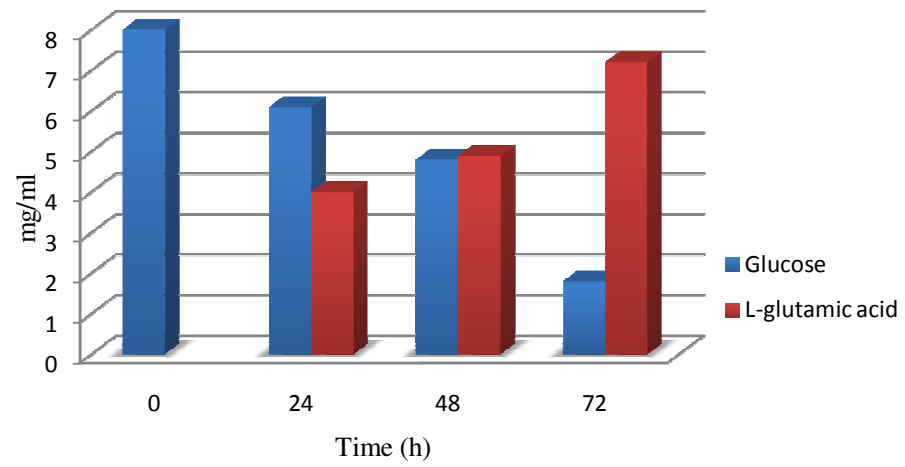

Fig 9 Changes in glucose and L- glutamic acid in pineapple waste by *Corynebacterium glutamicum* ATCC 13032

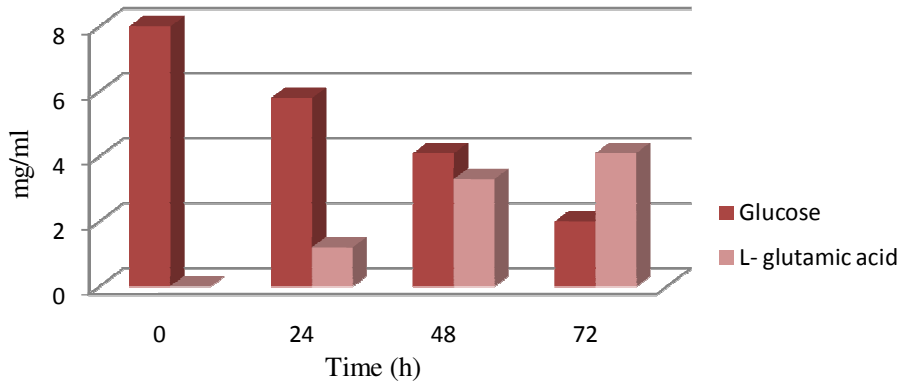

Fig 10 Changes in the glucose and L-glutamic acid production during fermentation of corn pomace hydrolysate by *B. subtilis* UG1

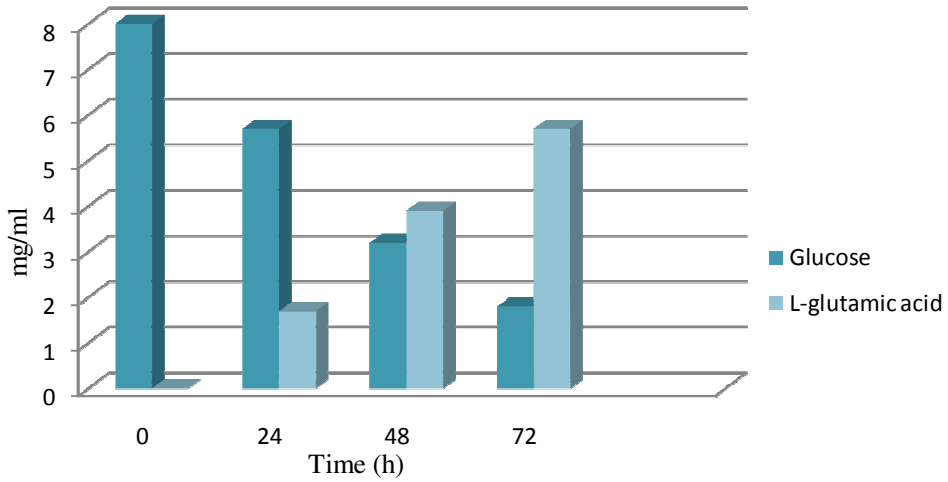

Fig 11 Changes in glucose and L-glutamic acid production during fermentation of corn pomace hydrolysate by B. pumilus DD4

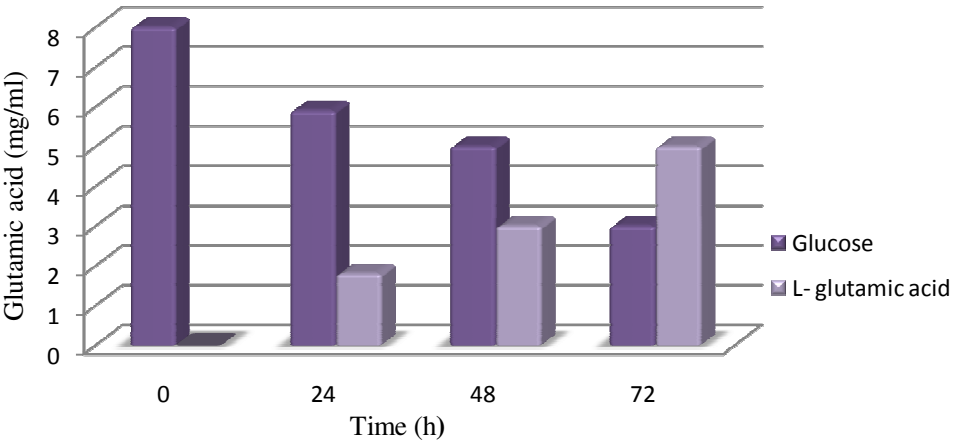

Fig 12 Changes in glucose and L-glutamic acid production during fermentation of corn pomace hydrolysate by *B. licheniformis* OG4

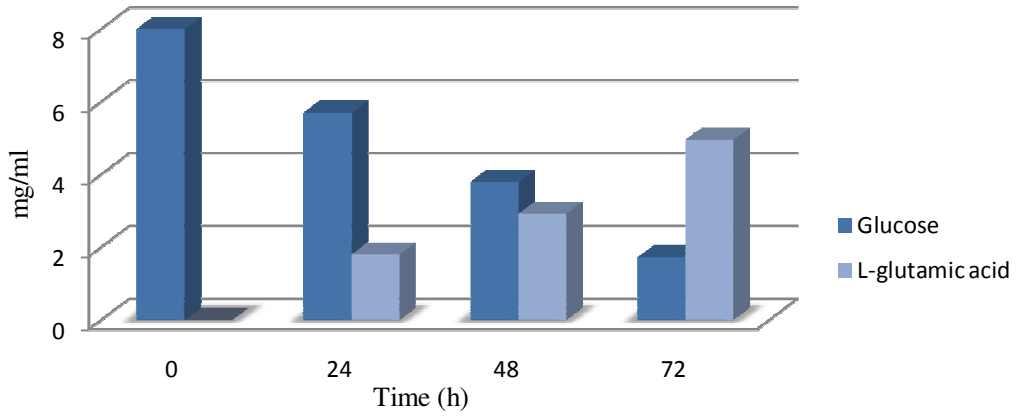

Fig 13 Changes in the glucose and L-glutamic acid production during fermentation of corn pomace hydrolysate by B.polymyxa OG7

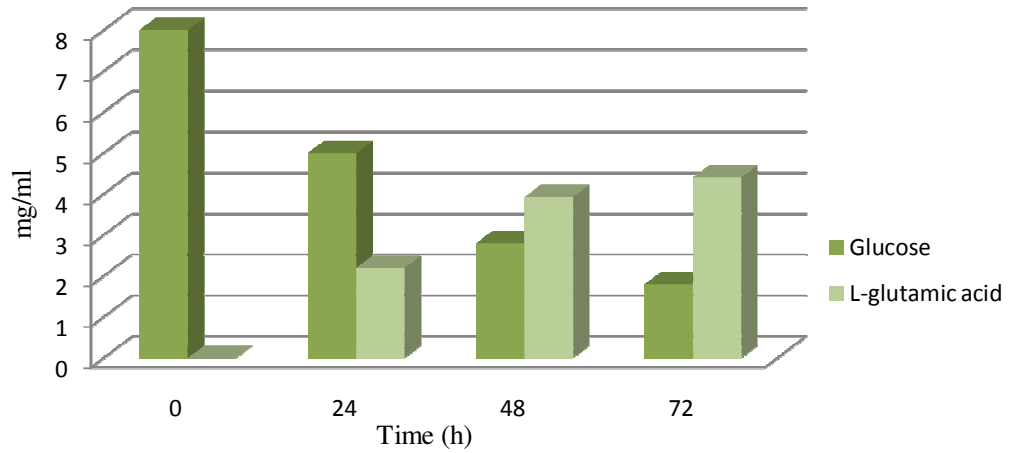

Fig 14 Changes in glucose and L-glutamic acid production during fermentation of corn pomace hydrolysate by Corynebacterium glutamicum ATCC 13032

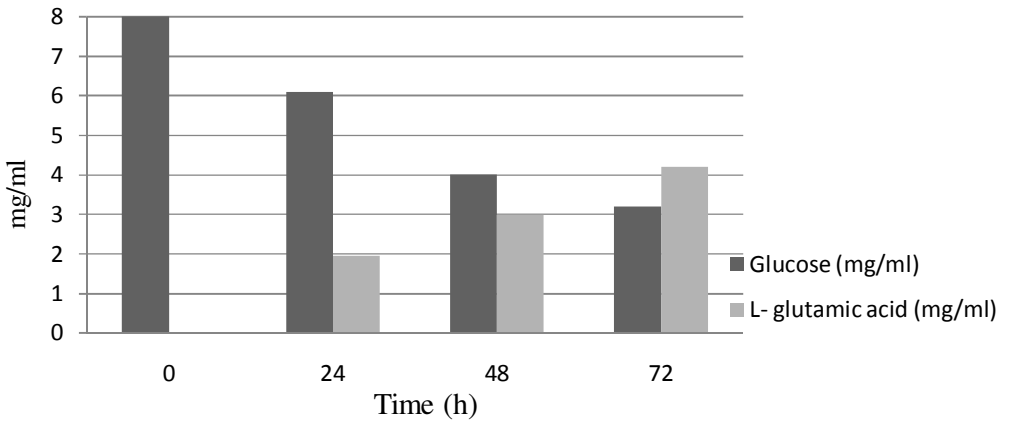

Fig 15 Changes in glucose and L-glutamic production during fermentation of cassava peel hydrolysate by B. subtilis.

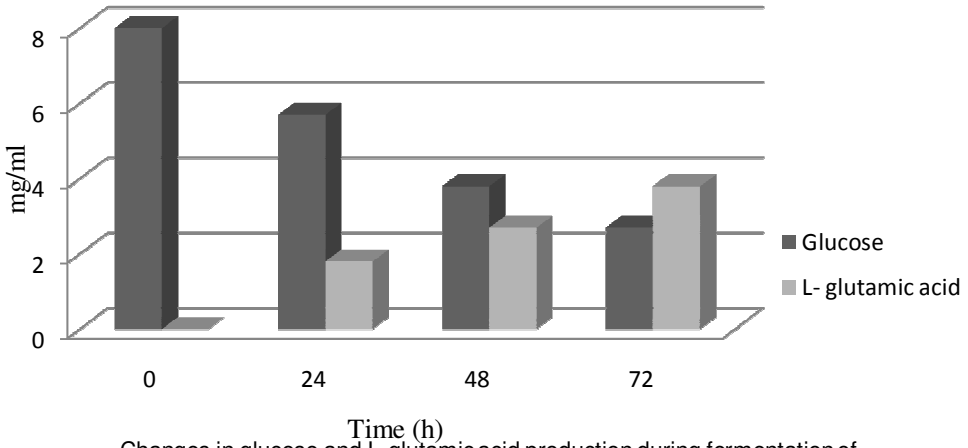

Fig 16 Changes in glucose and L-glutamic acid production during fermentation of cassava peel hydrolysate by B. pumilus DD4

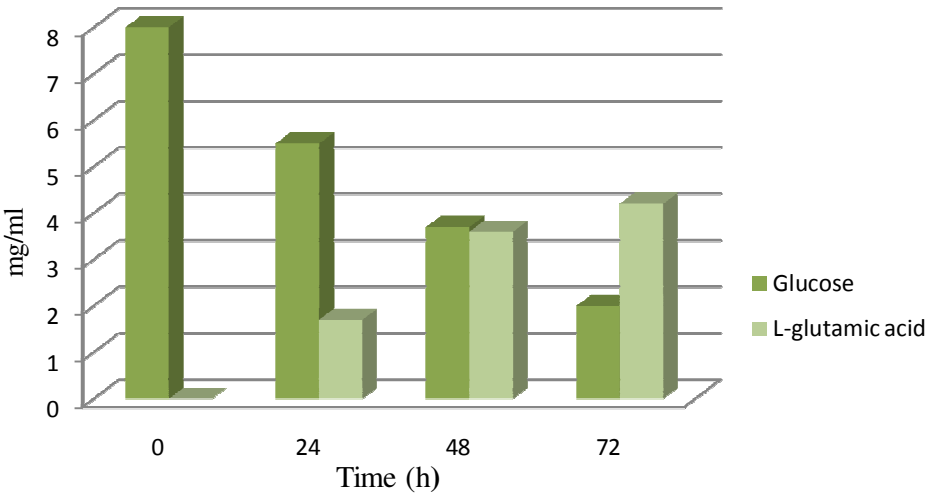

Fig 17 Changes in glucose and L-glutamic acid production during fermentation of cassava peel hydrolysate by B. licheniformis OG4

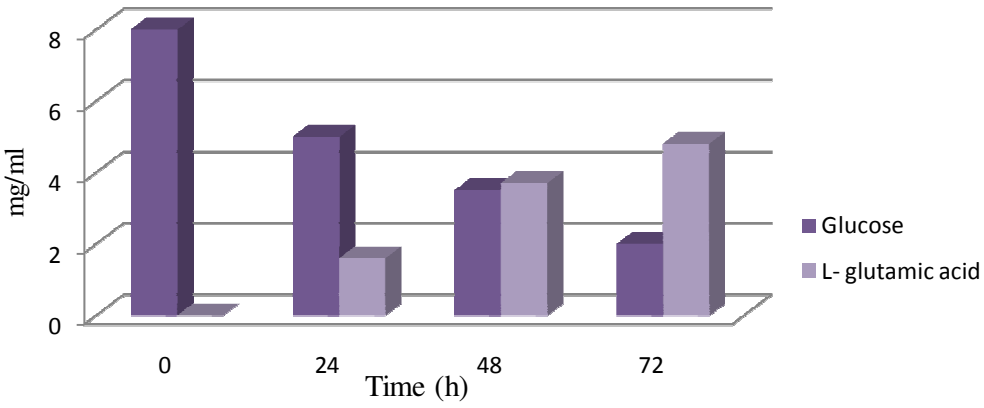

Fig 18 Changes in glucose and glutamic acid production during fermentation of cassava peel hydrolysate by *B.polymyxa* OG7

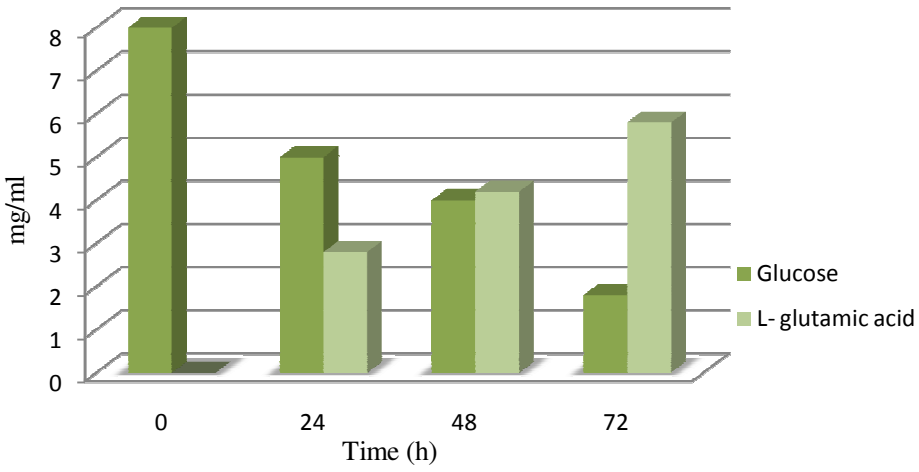

Fig 19 Changes in glucose and L-glutamic acid production during fermentation of cassava peel hydrolysate by Corynebacterium glutamicum ATCC 13032

Discussion

Previous studies carried out on L-glutamic acid by fermentation had been focused on the use of cassava starch hydrolysates and molasses as the raw materials (Hirose *et al.,* 1985). Cassava starch has a number of multi uses, which include popular fermented food products such as “gari” and “fufu” (Okafor and Ejiofor, 1986; Oyewole and Odunfa, 1990). Also corn could be used to achieve the endoamylase production of “ogi” (Banigo and Muller, 1972,Sahlin 1999,Oyetayo and Osho,2004).In order to prevent utilization of starches from the tubers of cassava and maize grains , attempts was made in the utilization of cassava peel and corn pomace which are wastes from these raw materials. Also, pineapple wastes, an abundant agricultural wastes materials from juice processors or when it had been eaten as snacks in its fresh state (Akpapunam *et al.,* 1993). Wastes material originating from pineapples, corn pomace, cassava peels are usually generated on a large scale without any known use. They constitute a high level of pollution to the environment and their quick evacuation from the environment because very necessary in order to prevent environmental pollution. Cassava peel and corn pomace contain about 42% starch which hydrolysis by starch degrading amylase enzyme yields between 70-80% fermentable sugars principally maltose and glucose (Oguntimein, 1993). Alpha amylase (α , 4-D- glucanglucano hydrolases E.C. 3.21.1 endoamylase) was produced from *Aspergillus niger* ATCC 16404 using rice bran by solid state fermentation. Alpha –amylase is widely distributed in micro-organisms, animals and plants (Oguntimein and safarik, 1993). The enzyme hydrolysis α, 1, 4 glycosidic bonds in amylose, amylopectin and glycogen in an endo fashion bringing about rapid decrease in the viscosity of starch solution.

Pineapple wastes contain a ready source of fermentable sugars.

Fermentable sugars from these wastes samples are used readily by the test isolates and the refrence strain *Corynebacterium glutamicum* ATCC 13032, as carbon source for growth and metabolic activities and hence glutamic acid production. Pineapples wastes were best utilized by the test isolates for growth and glutamic acid production and corn pomace was least utilized. The difference in the yield of glutamic acid might be as a result of the quick and rapid utilization of the fermentable sugars in the pineapple wastes than in the corn pomace. The rapid consumption of glucose was however made manifest in all the waste samples used for growth and L-glutamic acid production by the test isolate within 48 h of fermentation. However, after 72 h of fermentation, a sharp drop in pH to an acidic level due to the accumulation of glutamic acid.

The shift in the pH of the fermentation medium however lead to a fall in the production of L-glutamic acid. This result is in agreement with Chatopadhayay and Banerjee (1978), who also obtained ceasation in growth from 72 h of fermentation in a glucose medium. The entire fermentation process for the waste hydrolysates of cassava peel, corn pomace and pineapple wastes by the test isolate and the reference strain can be divided into two distinct phases.

The first phase (growth phase), active multiplication of the isolates took place with concurrent consumption of glucose and lowering of pH values. Glutamate production however began at 24 h. During the second phase (production phase) there was a slight increase in growth , glutamate accumulated and was excreted with a concomitant lowering of pH of the glucose initially present in the medium , nearly 40-50% was consumed during the first phase and another 20-30% during the second phase, leaving about 10% in the medium after fermentation was completed. The yields glutamate was 60% on the basis of glucose consumed. The shift of pH

towards acidity is one of the main causes for ceasation of growth (Hirose et al., 1985 Lawal *et.al* 2010).
Mutant will be of immense benefits and helps to expand the span of fermentation industry in the future through approaches to the pilot industrial purposes for L-glutamic acid production.
The present study has exploited the use of agro-industrial wastes of cassava peel, corn pomace and pineapple as potential substrates for L-glutamic acid and hence the pollutional effects produced through their indiscriminate disposal is thus prevented.
In future, application of DNA recombination techniques to the improvement of L-glutamic acid producing bacteria is another promising route.
Several kinds of plasmids relating to streptomycin resistance could be used as a possible vector system. Construction of a chimera plasmid involving a gene associated with L-glutamic acid biosynthesis could be also be performed .The possible clone of gene for starch hydrolysis and for L-glutamic acid could be packaged into a single strain and both functions exhibited at the same time instead of carrying out the function s separately.

CONCLUSION

This study has established that Agro-industrial wastes of cassava peel, corn pomace and pineapple wastes are suitable for the production of L- glutamic acid using organisms isolated from vegetable proteins. The organisms include *B.subtilis, B.licheniformis, B.polymyxa*, *B. pumilus.* The amount of L-glutamic acid production by them compared favourably with that produced by the reference strain *Corynebacterium glutamicum* ATCC 13032.

The use of agro-industrial waste for the production of L-glutamic acid will be of immense benefit to our food industries especially those that depend on flavour enhancing L- glutamic acid in their products. This will save these industries the huge foreign exchange which they currently spend on the importation of this item.

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