Biosynthesis of glutamic acid by immobilized *Pseudochrobactrum saccharolyticum* in a cowpea waste medium

Olaposi O Adegoke (adegokeolaposi@gmail.com)
Federal university of Agriculture
Sarafadeen O Kareem
Federal university of Agriculture
Saka A Balogun
Federal university of Agriculture
Abideen I Adeogun
Federal university of Agriculture

Research Article

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Abstract

It was tested whether bacteria immobilized on maize cob and corn husk might produce glutamic acid from cowpea waste. Glutamic acid synthesis was tested in bacteria isolates. Organic materials (corn cob and husk) were used as immobilization matrix and then used to produce glutamic acid. The effects of bead size, weight, and reusability were investigated. Glutamic acid production was best with a 2 mm bead size and a 3 kg bead weight. For the surface morphological view of the materials, a scanning electron microscope (SEM) was used. The samples were characterized using Fourier transform infrared (FTIR) analysis. The validity of a high-performance liquid chromatography (HPLC) method for the analysis of amino acids was determined, as well as the quantities of amino acids present. Immobilized *Pseudochrobactrum saccharolyticum* produced the highest amount of glutamic acid (9.4 g/L). The bacteria cells that had been immobilized were reused over and over again with no noticeable loss of activity. Nineteen amino acids were found, and these amino acids were separated by HPLC analysis. The outcome of the FTIR study revealed a large number of peaks, revealing the glutamic acid's complicated structure. This research found that bacteria immobilized on corn cobs and husks may successfully use lignocellulolytic materials to generate glutamic acid.

Introduction

Amino acids have been used as feed supplements, infusion compounds, medicinal agents, and precursors for the production of peptides and agrochemicals in the commercial world (Shyamkumar et al., 2014). The growing demand for monosodium glutamate as a flavor enhancer sparked interest in L-glutamic acid as the first amino acid to be generated on a wide scale through fermentation. It is now used as a flavor enhancer in meals all over the world in the form of monosodium salt (Ikeda, 2003). The monosodium salt of glutamic acid was then commercialized as monosodium glutamate (MSG) by Ajinomoto in the year (1909), earning it the popular trade name Ajinomoto. Since then, it has been utilized as a flavoring agent in a wide range of processed industry products in order to impact market economy (Ault, 2004; Alharbi et al., 2020). Several trials have been conducted to improve L-glutamic acid commercial production. Initially, protein hydrolysis was utilized, but it proved to be a time-consuming process. Later, chemical synthesis was attempted; however, throughout the process, a racemic mixture of DL isomers was produced, making isolation of the biologically active L-isomer of glutamic acid extremely difficult (Sano, 2009; Alharbi et al., 2020). It has various commercial values in addition to its ability to enhance flavor. It's a source of proline and arginine, among other amino acids. It functions as a neurotransmitter as well. As a result, excessive usage of monosodium glutamate as a flavor enhancer may result in neurotoxicity (Xiong et al., 2003). It has a variety of pharmacological and therapeutic applications. Glutamic acid and its derivatives are frequently utilized in the cosmetics business (Hermann 2003; Alharbi et al., 2020). The benefit of microbial L-glutamic acid production is that it yields just the optically active and physiologically needed L-form of glutamic acid (Amin and Al-Talhi, 2007). Glutamic acid bacteria include *Corynebacterium sp.*, *Brevibacterium sp., and Microbacterium sp.*, which are among the patent glutamic acid-producing strains by direct fermentation (Okamoto and Ikeda, 2000). Various

bacteria, such as Lactic acid Bacteria (Zarajen et al., 2012) and *Bacillus sp.*, have been found to produce glutamic acid in numerous investigations (Lawal et al., 2011). Bacteria that produce L-glutamic acid have been found in soil, sewage wastewater, vegetables, condiments, and aquatic settings (Shakoori et al., 2012, Lawal et al., 2011, Fodou et al., 2002). As a result, research has been conducted on the factors that influence glutamic acid production as well as yield (Lawal et al., 2011). When the fermentation media include modest quantities of biotin, optimal conditions for the synthesis of glutamic acid by Corynebacterium glutamicum are produced (Vijayalaskim and Sarvamangala, 2011). Microbial fermentation has been utilized to produce L-glutamic acid from a variety of carbon and nitrogen sources (Gupta et al., 2002).

In biotechnology, immobilization refers to the process of physically or chemically attaching cells, enzymes, or other proteins to a solid platform (Ahmed et al., 2008). The use of natural support materials for cell cross-linking has given immobilization matrices a new dimension (Osho et al., 2014). Reusability, non-toxicity, mechanical strength for necessary support, and open areas inside the matrix for cell growth are all advantages of such bio-structures (Akhtar et al., 2004). *Irvingia gabonensis, Detarium microcarpum* (Kareem et al., 2014), and Vegetable Sponge are some of the natural support matrices that have been documented so far (Osho et al., 2014).

Results

4.1 Isolation of bacteria

A total of eight (8) bacteria were isolated. All the isolates shows a positive result for catalase test, aesculin hydrolysis and methyl red, only one isolate shows a beta haemolysis while others were gamma haemolytic. Physiological and biochemical tests identified the bacteria as *Corynebacterium glutamicum sp*, *Bacillus flexus*, *Brevundimonas diminuta, Staphylococcus spp.*

Table i: showing the proximate analysis of acid and alkaline pretreated cowpea waste

Proximate composition	Acid treated cowpea waste (%)	Alkali treated cowpea waste (%)
Moisture content	13.33	14.54
Dry matter content	86.34	78.94
Fat content	10.12	8.53
Ash content	4.18	1.34
Crude fibre content	6.36	8.32
Crude protein content	46.82	47.48
Carbohydrate content	18.86	23.54

Table ii: Screening for L-Glutamic Acid Production by the organisms

Isolates code	Glutamic acid produced (g/L)
Staphylococcus spp	0.21
Pseudochrobactrum saccharolyticum	0.29
Bacillus cereus	0.35
Pseudochrobactrum saccharolyticum	0.39
Brevundimonas diminuta	0.45
Bacillus flexus	0.51
Pseudochrobactrum saccharolyticum	0.61

Discussion

4.2 Proximate Compositions of the Acid Pre-treated and Alkali Pre-treated cowpea waste

The acid-treated cowpea had a greater ash level of 4.18 percent, while the alkali-treated cowpea had just 1.34 percent, according to proximate analyses of both the acid and alkali-treated dried unfermented cowpea waste samples. The alkali-treated cowpea, on the other hand, had a greater carbohydrate content of 23.54 percent, compared to 18.86 percent for the acid-treated cowpea. This could be related to the fact that, according to (Damisa et al., 2008) acid pretreatment of residues normally does not eliminate lignin from the substrate, but only alters the lignin CHO linkage. This is also in line with the findings of (Houghton et al., 2006), who claimed that acidic substrate treatment could allow cellulose to re-anneal, resulting in horrification of cellulose into micro fibrils instead. Moisture content, crude fat, crude protein, and crude fiber content were also determined, as indicated in Table i.

4.3. Screening for glutamic acid producing bacteria

All of the microorganisms studied produced glutamic acid, which was qualitatively identified using paper chromatography. Table ii shows that among the seven isolates proved to be *Pseudochrobactrum saccharolyticum* and screened for L-glutamic acid synthesis, the isolate from the chicken-pen produced the highest glutamic acid yield of 0.61g/L, while the isolate from the sheep-pen produced the lowest glutamic acid yield of 0.21g/L. This observed disparity in glutamic acid yield could be attributed to *Pseudochrobactrum saccharolyticum s* nutritional diversity's variety and adaptation, which is usually associated with the nature of the environment from which they were previously separated. This study's maximum glutamic acid production of 0.61g/L is significantly lower than (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 10 µg used in this study as opposed to the lower concentration of 50 µg used by (Hadia et al., 2012).

4.4.0 Scanning Electron Microscope

In this work, SEM was used to examine the material's surface morphology in order to determine whether CC has enough carbonaceous material to be acceptable for gasification utilizing a downdraft gasification system. The data was collected across a specific area of the sample's surface, and a two-dimensional image was created, as shown in Plates A1, A2 and B1, B2 that indicated spatial changes in characteristics.

4.4.1. FTIR analysis of the result showed that a lot of numbers of peaks were detected, informing the complex structure material of corn cob and corn husk in figure i and ii

4.4.2 Effect of bead size on glutamic acid production from cowpea waste using corn cob and corn husk matrix

Figure i and ii shows the production of glutamic acid from cowpea waste by bacteria immobilized in corn cob and husk beads with diameters of 1, 2, 3, and 4 mm.

The volume of glutamic acid produced increased gradually as the bead size increased from 1 mm to 2 mm, then decreased as the bead size increased (Fig. iii and fig iv). As shown in Figures iii and iv, the bead size 2 mm produced the highest volume of glutamic acid, indicating that the number of pore spaces made available and the number of cells filling each space are optimal for substrate consumption (Kareem et al., 2013). Larger sizes resulted in smaller beads, indicating that smaller beads had more surface area per unit volume and thus higher productivity (Kareem et al., 2014). Because the cells in larger beads have less access to the substrate, it reacts with the molecules and produces a product (Sevda and Rodrigues, 2011). Using a corn cob matrix, immobilized *Pseudochrobactrum saccharolyticum* produced (9.4 g/L) from acid treated cowpea waste and (8.2 g/L) from alkaline treated cowpea waste, whereas a corn husk matrix produced 7.8 g/L and 6.2 g/L from acid and alkaline treated cowpea waste, respectively. This could be because they are biotin auxotrophs who release L-glutamic acid in response to biotin deficiency. *Pseudochrobactrum saccharolyticum* is a glutamic acid-producing bacteria, according to (Ahmed et al., 2008).

4.4.3 Effect of bead weight on glutamic acid production from cowpea waste using corn cob matrix

Production of glutamic acid from cowpea waste by bacteria immobilized in 1, 2, 3 and 4 kg diameter corn cob beads is shown in Fig. iii and iv. There was a gradual increase in volume of glutamic acid produced with bead size from 1 kg to 3 kg and thereafter decrease with increase in bead weight (Fig. v and fig vi). As illustrated in Fig. v and vi, highest volume of glutamic acid produced was obtained with bead weight 3 kg, which indicate that this weight is maximum for substrate utilization.

4.4.4 Effect of bead reusability on glutamic acid production from cowpea waste using corn cob matrix

Figures vii and viii show the effect of using immobilized bacteria repeatedly on the generation of glutamic acid from cowpea waste. The results demonstrated that bacteria cells that had been immobilized may be employed again and again without losing their activity. During cell re-use, it was discovered that immobilized *Pseudochrobactrum saccharolyticum* produced the most glutamic acid from cowpea waste. For the acid treated cowpea waste (Fig. vii), it retained 66 percent, 53 percent, and 35 percent glutamic acid yield for the second, third, and fourth cycles, respectively, and for the alkaline treated cowpea waste (Fig. vii), it retained 58 percent, 46 percent, and 32 percent glutamic acid yield for the second, third, and fourth cycles, respectively. Similarly For the acid treated cowpea waste (Fig. viii), it retained 69 percent, 45 percent, and 30 percent glutamic acid yield for the second, third, and fourth cycles, respectively, and for the alkaline treated cowpea waste (Fig. viii), it retained 54 percent, 40 percent, and 30 percent glutamic acid yield for the second, third, and fourth cycles, respectively. This is marginally better than the findings of (Anwar et al., 2009) who discovered that immobilized cells retained 30% of their original activity. This could be due to blockage of cells within the matrix after the sixth cycle (Kareem et al., 2013).

4.4.5 HPLC analysis

HPLC was used to determine the quantitative concentration of amino acids, and the amino acid profile is depicted in Figure ix (a and b). There are seventeen amino acids found in the sample, and their separation was reasonably resolved. Methionine, leucine, lysine, cysteine, phenylalanine, tyrosine, arginine, isoleucine, threonine, and valine were discovered to be present, along with seven non-essential amino acids.

Materials And Methods

2.1 Collection of samples

A total of two different soil locations at Camp, Abeokuta were considered for sample collection to isolate *Pseudochrobactrum saccharolyticum*. Two samples each from chicken and sheep pen

2.2. Substrates

Cowpea waste was bought from a market at Osiele in Abeokuta, Ogun State. The cowpea waste sample was packaged into polythene bag, labeled appropriately and then transported to the Department of Microbiology, Federal University of Agriculture, Abeokuta for analyses.

2.3. Treatment of the Cowpea waste (Substrate Pre-treatment)

Cowpea waste were separately pre-treated using sodium hydroxide and sulphuric acid and were used as substrate for fermentation to glutamic acid production.

2.3.1. Alkaline Pre-treatment

In a 1000ml Erlenmeyer flask, 25 g of fresh cowpea waste were weighed and 225 ml of 3 percent (w/v) NaOH solution were added. The flask was cotton-plugged and autoclaved for 30 minutes at 121°C. After treatment, the material was filtered through muslin cloth and washed many times in distilled water until no color was apparent in the wash water and the pH was corrected to a physiological level (7.2). The neutralized residue was manually squeezed to eliminate excess water before being used for enzymatic hydrolysis and glutamic acid fermentation. At the Department of Microbiology, Federal University Of Agriculture, Abeokuta, a small amount of the treated biomass was dried in an oven at 70°C for 24 hours and ground to fine particle size in a laboratory mill for compositional (proximate) analysis studies (Rakesh and Devendra, 2013).

2.3.2 Acidic Pre-treatment

The approach provided by (Rakesh and Devendra, 2013) for acidic pretreatment was used. A 1000ml flask was filled with around 25 g of cowpea waste, as well as roughly 225ml of 3 percent sulphuric acid. Following calcium hydroxide over-liming, the mixture was autoclaved at 121°C for 30 minutes, and the material produced after treatment was filtered through muslin cloth and washed multiple times under running distilled water until no color was apparent in the wash water. The neutralized residue was manually squeezed to eliminate excess water before being used for enzymatic hydrolysis and glutamic acid fermentation. For the compositional (proximate) analysis experiments, a small sample of the treated biomass was dried in an oven at 70°C for 24 hours and ground to fine particle size in a laboratory mill (Devendra and Rakesh, 2013) at the Department of Microbiology, Federal University Of Agriculture, Abeokuta.

2.3.3 Proximate Analyses of the Sample

Approximately 8 g of each acid-treated and alkali-treated dried unfermented cowpea waste sample was used to conduct proximate analyses at the Department of Microbiology, Federal University Of Agriculture, Abeokuta, in order to determine the total carbohydrate content, crude protein content, crude fat, crude fiber, ash content, and moisture content as percentage compositions of the substrate using the methods proposed by (Ahmed et al., 2013).

2.4. Isolation and characterization of bacteria

2.4.1. Isolation of glutamic acid producing bacteria

2.4.2 Isolation of *Pseudochrobactrum saccharolyticum*

A weighted amount of 1g of soil sample was added to 10 ml of sterile distilled water and a tenfold serial dilution using sterile normal saline as diluent was carried out to a dilution of 10 − 5. By streaking, a loopful (0.1ml) of each of the $1:10^2$ diluted soil suspensions was inoculated onto slants of Loeffler's medium, which served as the major isolation medium. The inoculated slants were kept at 35°C for 48 hours after being inoculated. Colonies that were discrete and well isolated were chosen and identified. These were sub-cultured on modified Hoyle's selective medium and incubated at 35°C for 48 hours. The

isolates were employed as a source of culture to test for L-glutamic acid synthesis (Ahmed et al., 2013). To identify and use the isolates, they were sub-cultured onto nutrient agar slants.

Preliminary Screening Of The Isolates For Glutamic Acid Production

3.1. Screening Medium

The screening medium for L-glutamate production contains the following composition per 1000 ml of dH2O: glucose, 5 gm; calcium carbonate, 1 gm; ammonium sulphate, 1 gm; potassium di-hydrogen phosphate, 0.3 gm; di-potassium hydrogen phosphate, 0.7 gm; magnesium sulphate hepta-hydrate, 0.2 mg ; thymine hydrochloride, 0.01 gm; ferrous sulphate heptahydrate, 0.2 mg; magnesium chloride tetra-hydrate, 20µg; and d-biotin, 10 µg.

3.2. Culture Procedure

Each 250 ml flask was filled with 100 mL of screening medium, sterilized and inoculated with a 24-hour-old bacterial broth culture. The flasks were shaken at 180 rpm for 96 hours at 37°C in a shaker incubator. After 96 hours, samples were taken and centrifuged for 10 minutes at 5000 rpm. The presence of L-glutamic acid in the supernatants was then determined.

3.3. Qualitative Estimation of Glutamate

The paper chromatographic technique was used for qualitative analysis of L-Glutamate, as stated by (Hassan et al., 2003).

3.4. Quantitative Estimation of Glutamate

Two milliliters of the supernatant from each fermented screening media was taken individually in test tubes, two milliliters of 5% ninhydrin in acetone was added, and the test tubes were cooked in a boiling water bath for 15 minutes. The tubes were then cooled to room temperature, and glutamate was quantified using a spectrophotometer to take values at 570 nm.

3.5. Immobilization of bacteria

In this investigation, bacteria suspension was immobilized using maize cob and corn husk as carriers. These materials were first broken down into smaller pieces. The pieces were then soaked in distill water for 5 to 6 hours to eliminate any remaining miscellaneous, and then cleaned twice or three times with alcohol and distilled water. The pieces were dried for 2 hours at 105°C. Following that, these fragments were utilized as carriers to immobilize bacteria (Liu Zhixiu et al., 2017).

3.6. Optimization studies of glutamic acid production

Glutamic acid production was carried out using the basal salt medium containing 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, $ZnSO_4 \cdot 7H2O$; 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 $NaMo_2O_4 \cdot 2H_2O$; 200 µg of biotin (pH 7.0). The initial pH was adjusted at 7.2 with 1N NaOH and 6% cowpea waste. The medium was incubated at 30^oC for 96 hrs. Glutamic acid production from immobilized bacteria was optimized using different parameters such as bead size, bead weight and bead reusability.

3.6.1 FTIR analysis

Samples are exposed to infrared (IR) radiation during the FTIR analysis technique. The IR radiations then interact with the atomic vibrations of a molecule in the sample, resulting in energy absorption and/or transmission specific to that molecule. As a result, the FTIR can be used to identify individual molecular vibrations in a material (De Meutter et al., 2016).

3.6.2 HPLC analysis

A reverse phase Pico-Tag column (3.9 x 300mm) C18 at 400 C and a UV detector at 254 nm were used for chromatographic separation of the hydrolysates. Two eluents, (A) an aqueous buffer and (B) 60 percent acetonitrile in water, made up the solvent system. Two pumps, set to provide the mobile phases eluents A and B, were used to perform gradient elution. A gradient was used for the separation, which comprised of 10% B travelling to 51% B in 10 minutes utilizing a convex curve (number 5). Each set of experimental samples was compared to a set of amino acid standards (Merck Germany). The amino acids in the samples were identified by comparing their retention durations to those of the standards (Cui et al., 2013)

3.6.3 Statistical analysis

The data were processed using a statistical approach using the SPSS 13.0 software, Wagner (2019). All optimization parameters were compared using One-Way ANOVA.

Conclusion

From the study, it was concluded that *Corynebacterium glutamicum* was successfully immobilized on corn cob and corn husk and this bacteria can directly produce glutamic acid from cowpea waste through a single-step process.

Declarations

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics approval

“This is an observational study. The study Research Ethics Committee has confirmed that no ethical approval is required.”

Consent to participate

“Informed consent was obtained from all individual participants included in the study.”

Consent to publish

“The authors affirm that no human research participants were provided

Data availability

No data was used for the research described in the article..

Code availability

Not applicable

Declarations Contribution of the Author

Adegoke Olaposi was in charge of conducting the experiments, analyzing and interpreting the results, and writing the paper. Kareem Sarafadeen: Conceived and planned the experiments; provided reagents, materials, analytical tools, or data; Balogun Saka: Analyzed and interpreted data; provided reagents, materials, analysis tools, and data. Adeogun Abideen: Analyzed and interpreted data; provided reagents, materials, analysis tools, or data

Competing Interests

“The authors have no relevant financial or non-financial interests to disclose.”

Human Participation

No human participation involved in this research and no data material collected in the course of the research

Animal Participation

No animal participation involved in this research and no data material collected in the course of the research

Utilization of Bacteria:

Corynebacterium glutamicum was isolated and used in the course of this research

Author Contributions

“All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Adegoke Olaposi], [Kareem Sarafadeen], [Balogun Saka] and [Adeogun Abideen]. The first draft of the manuscript was written by [Adegoke Olaposi] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

References

1. Ahmed, S.A., Ramadan, A.A., Domany, N.M., Hesham, H.R., Shireen, A.S. Optimization, immobilization of extracellular alkaline protease and characterization of its enzymatic properties. Res. J. Agric. Biol. Sci. 4 (5), 434–446 (2008).
2. Akhtar, N., Iqbal, J., Iqbal, M. Removal and recovery of nickel (II) from aqueous solution by loofa sponge-immobilized biomass of Chlorella sorokiniana: characterization studies. J. Hazard Mater. 108, 85–94 (2004).
3. Alharbi NS, Kadaikunnan S, Khaled JM. Optimization of glutamic acid production by Corynebacterium glutamicum using response surface methodology. Journal of King Saud University – Science vol 32 No 2 Pages. doi.org/10.1016/j.jksus.2019.11.034 (2020).
4. Amin GA, Al-Talhi A. Production of L-glutamic acid by immobilized cell reactor of the bacterium Corynebacterium glutamicum entrapped into carrageenan gel beads. World Appl Sci J; 2(1):62–67 (2007).
5. Anwar, A., Qader, S., Raiz, A., Iqbal, S., Azhar, A. Calcium Alginate: a support material for immobilization of proteases from newly isolated strain of Bacillus subtilis KIBGE-HAS. World Appl. Sci. J. 7 (10), 1281–1286 (2009).
6. Ault, A. The monosodium glutamate story: the commercial production of MSG and other amino acids. J. Chem. Educ. 81, 347 (2004).
7. Cui J.D., Li L.L., Bian H.J., (2013). Immobilization of cross-linked phenylalanine ammonia lyase aggregates in microporous silica gel. PLoS ONE; 8(11):e80581
8. Damisa, D., Ameh, J.B. and Umoh, V.J. Effect of Chemical Pretreatment of Some Lignocellulosic Wastes on the Recovery of Cellulase from Aspergillus niger AH3 Mutant. African Journal of Biotechnology, 7(14):2444–2450 (2008).
9. De Meutter J, Derfoufi MK, Goormaghtigh E. Analysis of pro-tein microarrays by FTIR imaging. Biomed Spectrosc Imaging 5:145–154 (2016).
10. Fodou, R., Jojim,a Y., Seto, A., Yamada, K., Kimura, E., Nakamatsu, T., Hiraishi, A. and Tamanaka, S. Corynebacterium efficiens sp. nov., a glutamic acid producing species from soil and vegetables. Int. J. Syst. Evol. Microbiol. 52: 1127 – 1137 (2002).
11. Gupta, R., Beg, Q.K and Lorenz, P. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Microbiol. & Biotechnol, 59: 15–32 (2002).

12. 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
13. Hassan, B., Asghar, M., Nadeem, S., Zubair, H., Muzammil, H.M. and Shahid, M. Isolation and Screening of Amino acids-Producing Bacteria from Milk. Biotechnology, 2(1):18–29 (2003).
14. Hermann T., (2003). Industrial production of amino acids by coryneform bacteria. J Biotechnol, 104(1–3):155–172.
15. Houghton, J., Weatherwax, S. and Ferrell, J., (2006). Breaking the Biological Barriers to Cellusosic Ethanol: a Joint Research Agenda. A Research Road Map Resulting from the Biomass to Biofuels Workshop Sponsored by the Office of Science and Office of Energy Efficiency and Renewable Energy, US Dept of Energy.
16. Kareem, S.O., Oladipupo, I.O., Omemu, A.M., Babajide, J.M. Production of citric acid by Aspergillus niger immobilized in *Detarium microcarpum* matrix. Malaysian J. Microbiol. 9 (2), 161–165 (2013).
17. Kurilich A.C, Tsau G.J, Brown A, Howard L, Klein B.P. Carotene, tocopherol and ascorbate content in subspecies of *Brassica oleracea*. J Agric Food Chem 47(4): 1576–1581 (1999).
18. Lawal, A.K., Oso, B.A., Sanni, A.I and Olatunji, O.O. L-glutamic acid production by Bacillus spp. Isolated from vegetable proteins. Afr. J. Biotechnol., 10(27): 5337–5345 (2011).
19. Liu Zhixiu, et al. China Petroleum Processing and Petrochemical Technology, 19(2): 33–39 (2017).
20. Okamoto, K. and Ikeda, M. Development of an industrial stable process for L – threonine fermentation by an L- methionine –auxotrophic mutant of Escherichia coli. J. Biosci. Bioeng., 89: 87–89 (2000).
21. Osho, M.B., Popoola, T.O., Kareem, S.O., Arowolo, T.A. Transesterification of Jatropha seeds oil by vegetative sponge immobilized lipase of Alternaria sp. MGGP 06 for fatty acid methyl ester production under optimized conditions. Pet. Technol. Dev. J. 1, 56–70 (2014).
22. Rakesh, K.T. and Devendra, P.S. 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 (2013).
23. SANO, C. Histrory of glutamate production. Am J Clin Nutr., 90:728S-732S (2009).
24. Sevda, S.B., Rodrigues, L. The making of pomegranate wine using yeast immobilized on sodium alginate. Afr. J. Food Sci. 5 (5), 229–304 (2011).
25. Shakoori, F.R., Ali, N.M and Shakoori, A.R. Improvement in cysteine production by local bacterial isolates. Pakistan J. Zool., 43: 805–808 (2012).
26. Shyamkumar, R., Moorthy, I.M.G., Ponmurugan, K., Baskar, R. Production of L-glutamic acid with Corynebacterium glutamic (NCIM 2168) and Pseudomonas reptilivora (NCIM 2598): a study on immobilization and reusability. Avicenna J. Med. Biotechnol. 6, 163 (2014).
27. Vijayalakshim P and Sarvamangala D. Production of L-glutamic acid by Cornynebacterium glutamicum DSM 20300T and Arthrobacter globiformis MTCC 4299 using fruits of Muntingia. Int. Res. J. Microbiol., 2:116–121 (2011).

28. Wagner III, W. E. Using IBM® SPSS® statistics for research methods and social science statistics. Sage Publications (2019).
29. Xiong Z.G., Zhu X.M., Chu X.P., Minami M., Hey J., Wei W.L., MacDonald J.F., Wemmie JA., Price M.P., Welsh M.J., Simon R.P. Neuroprotection in ischemia: blocking calcium-permeable Acid-sensing ion channels. Cell; 118(6):687–698 (2004).
30. Zarejan M., Ebrahimpour A., Abu Bakar F., Sabo Mohamed, A., Forghani B., Ab- Kadir M. S. and Saari, N. Glutamic Acid Producing Lactic Acid Bacteria Isolated from Malaysian Fermented Foods. Int. J. Mol. Sci., 13(5): 5482–5497 (2012).

Figures

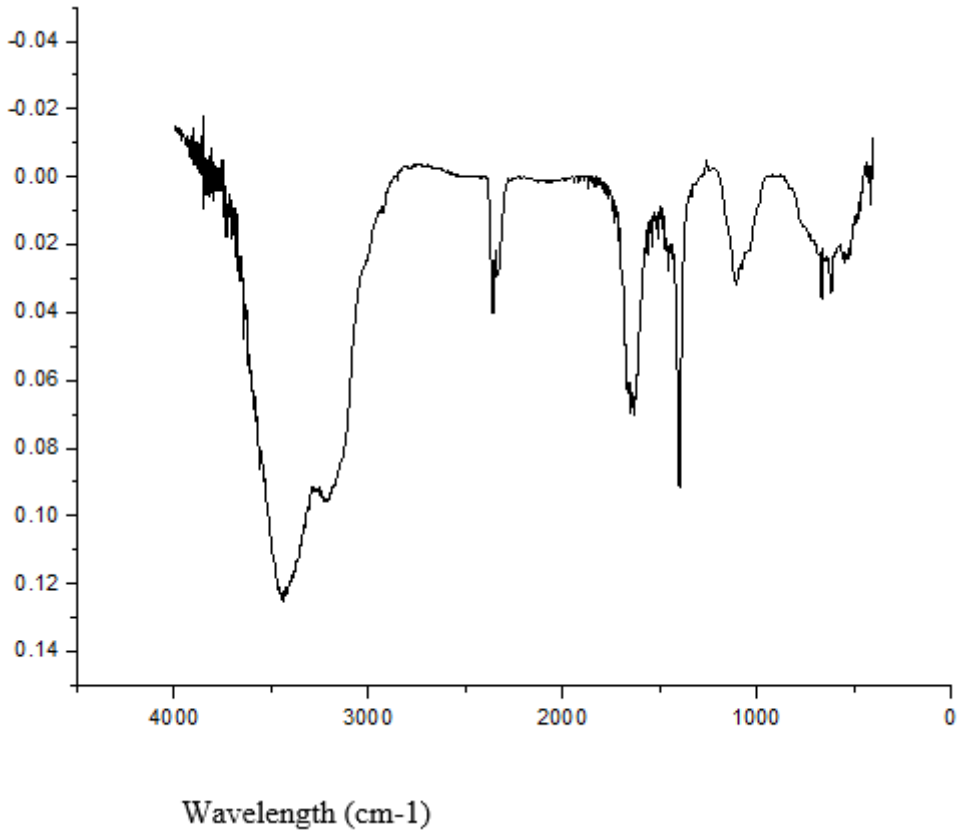


Figure 1

FT-IR for corn cob

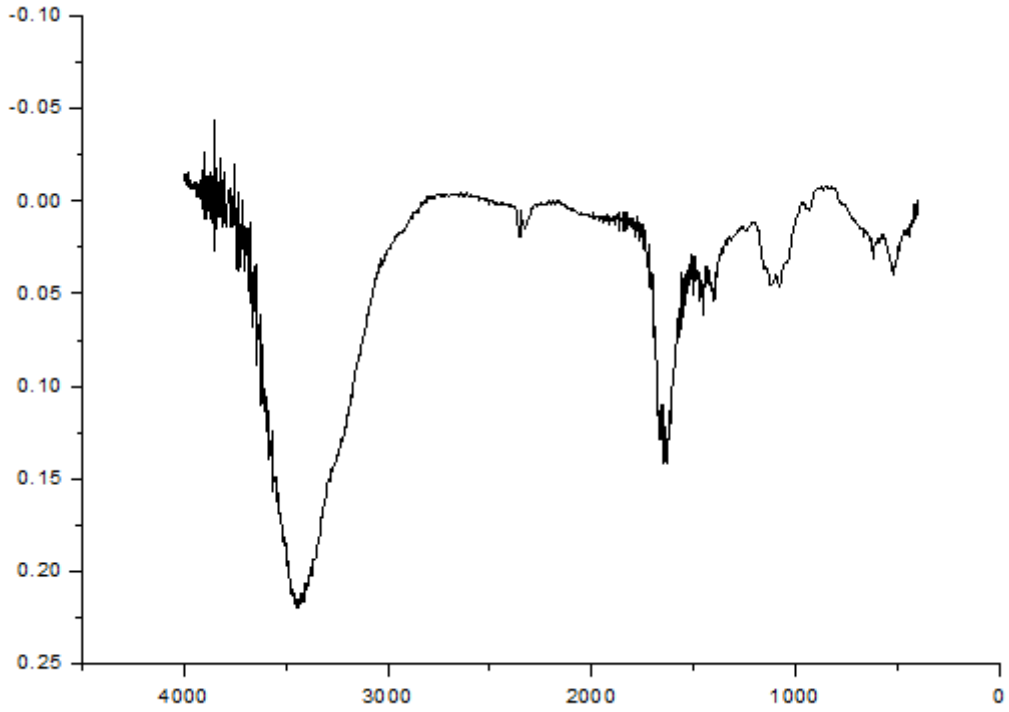


Figure 2

FT-IR for corn husk

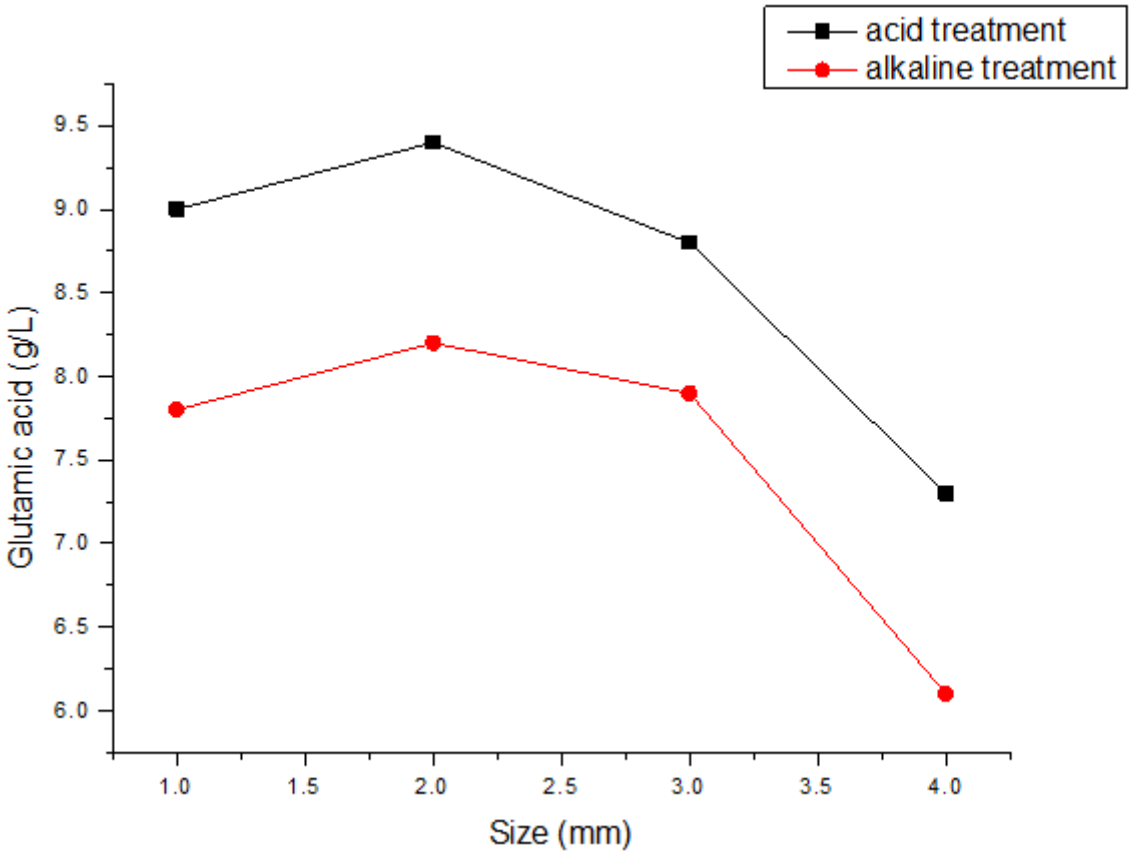


Figure 3

Effect of bead size on glutamic acid production from cowpea waste

NB: Corn cob matrix (acid and alkaline treated cowpea waste)

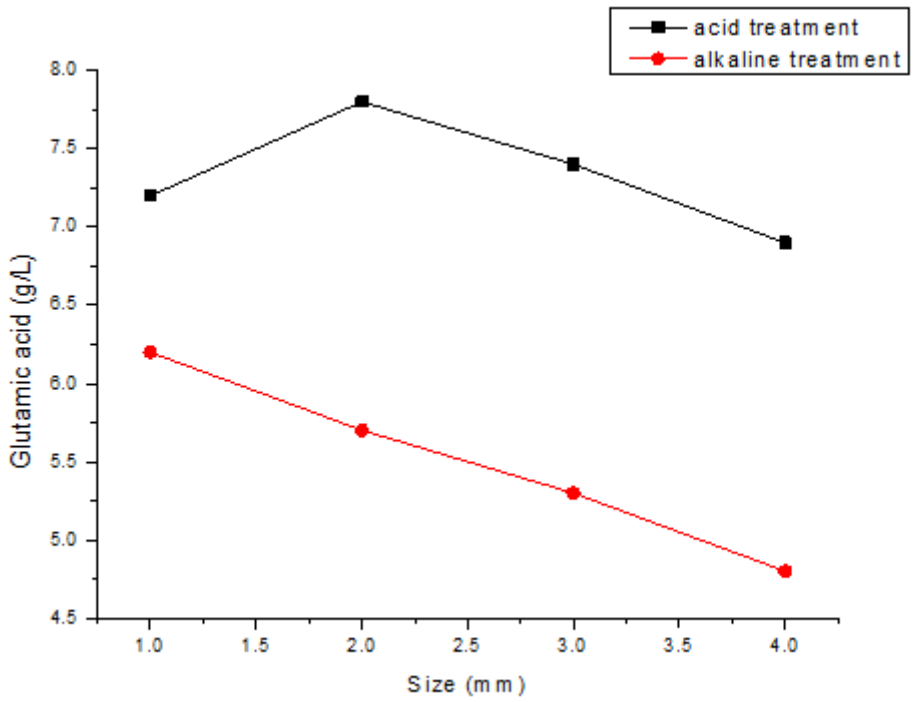


Figure 4

Effect of bead size on glutamic acid production from cowpea waste

NB: Corn husk matrix (acid and alkaline treated cowpea waste)

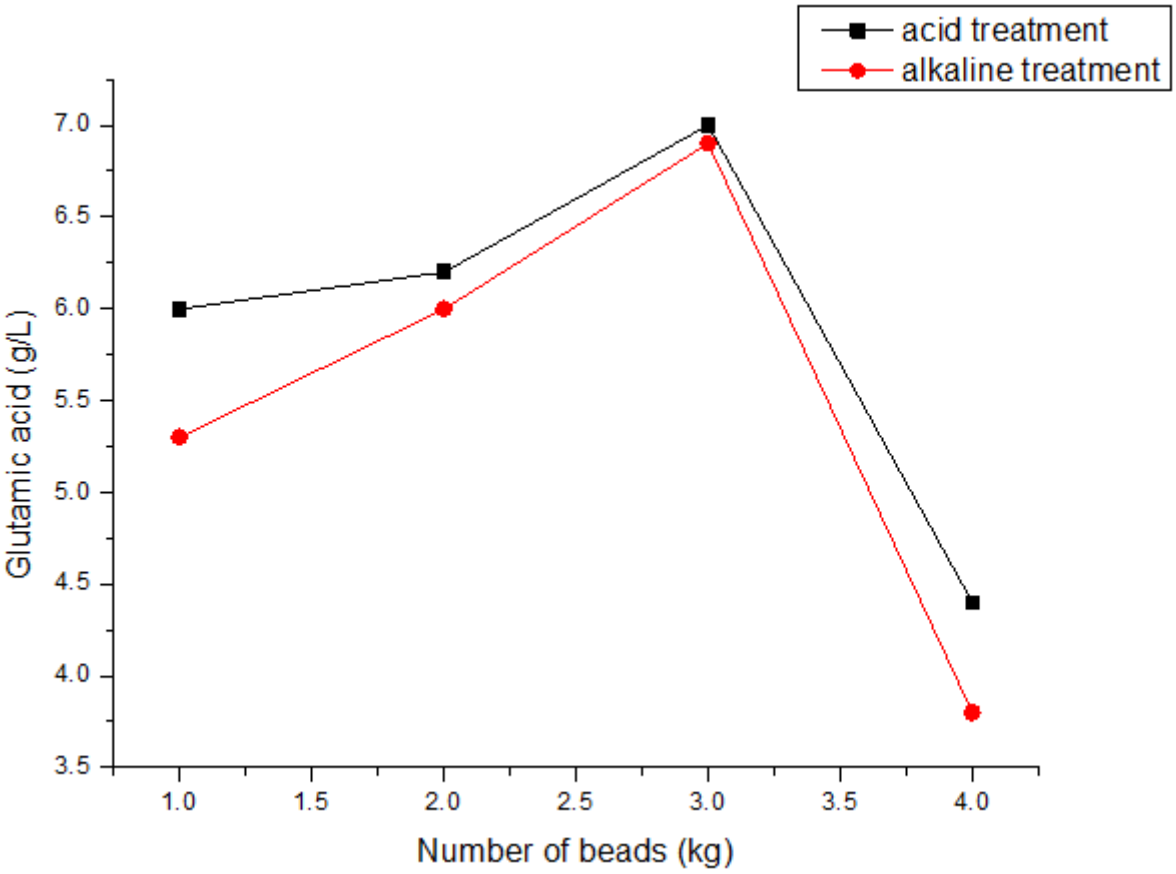


Figure 5

Number of bead on glutamic acid production from cowpea waste

NB: Corn cob matrix (acid and alkaline treated cowpea waste)

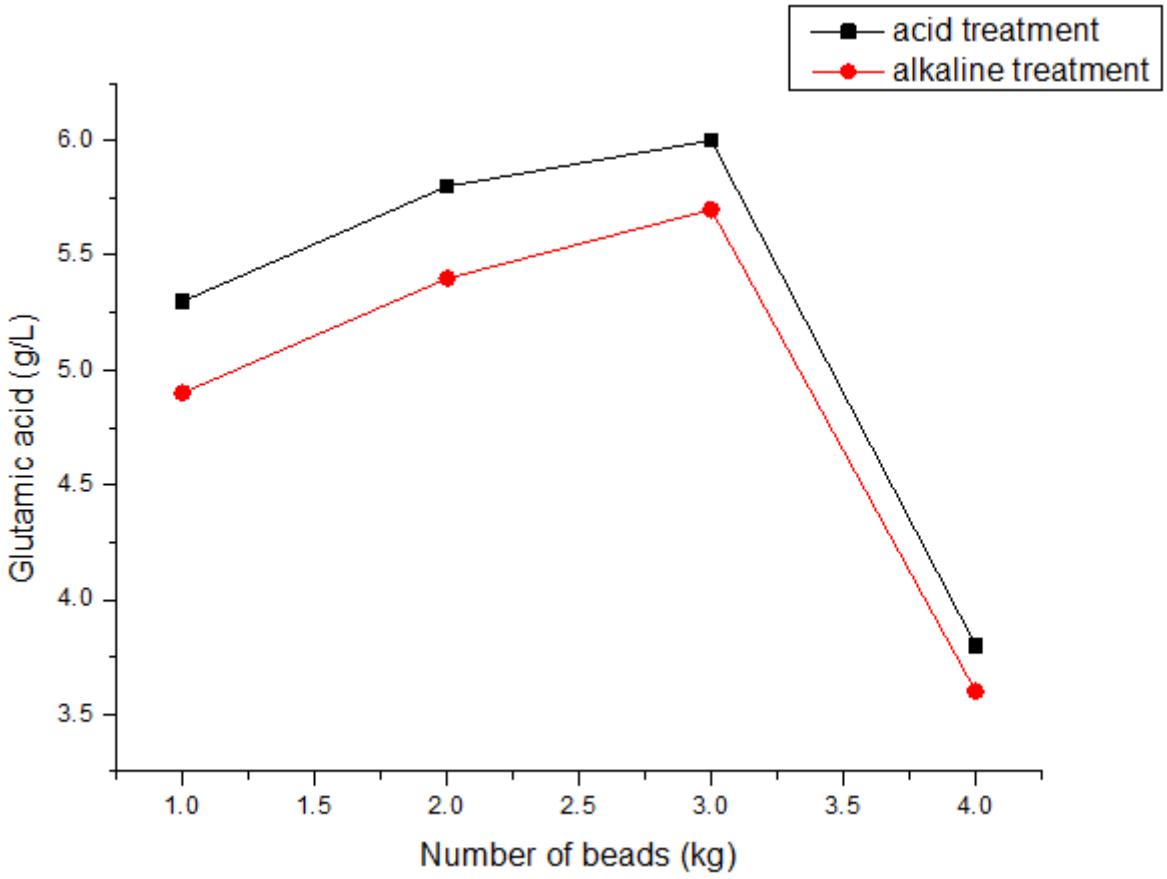


Figure 6

Number of bead on glutamic acid production from cowpea waste treatment

NB: Corn husk matrix (acid and alkaline treated cowpea waste)

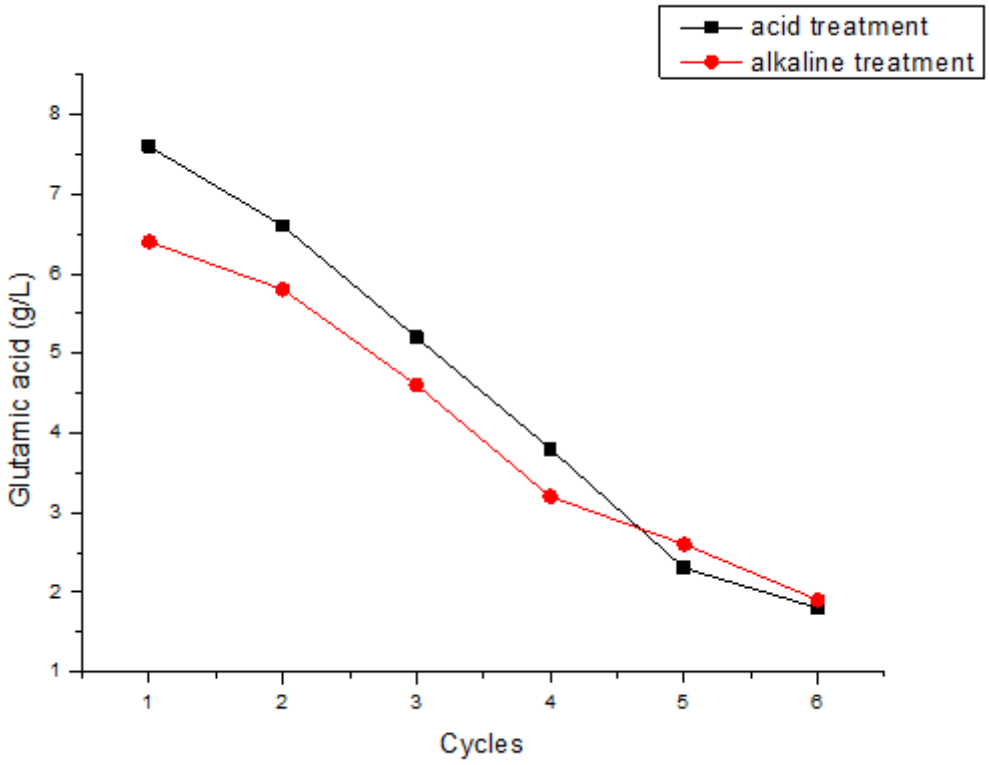


Figure 7

Effect of bead reusability on glutamic acid production from cowpea waste

NB: Corn cob matrix (acid and alkaline treated cowpea waste)

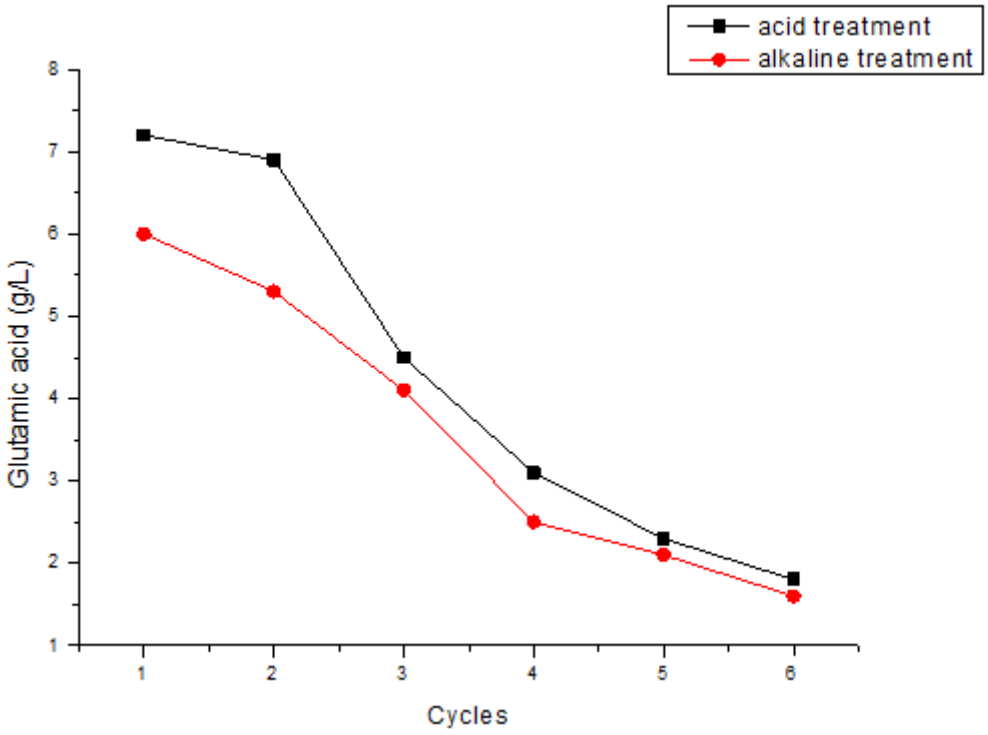
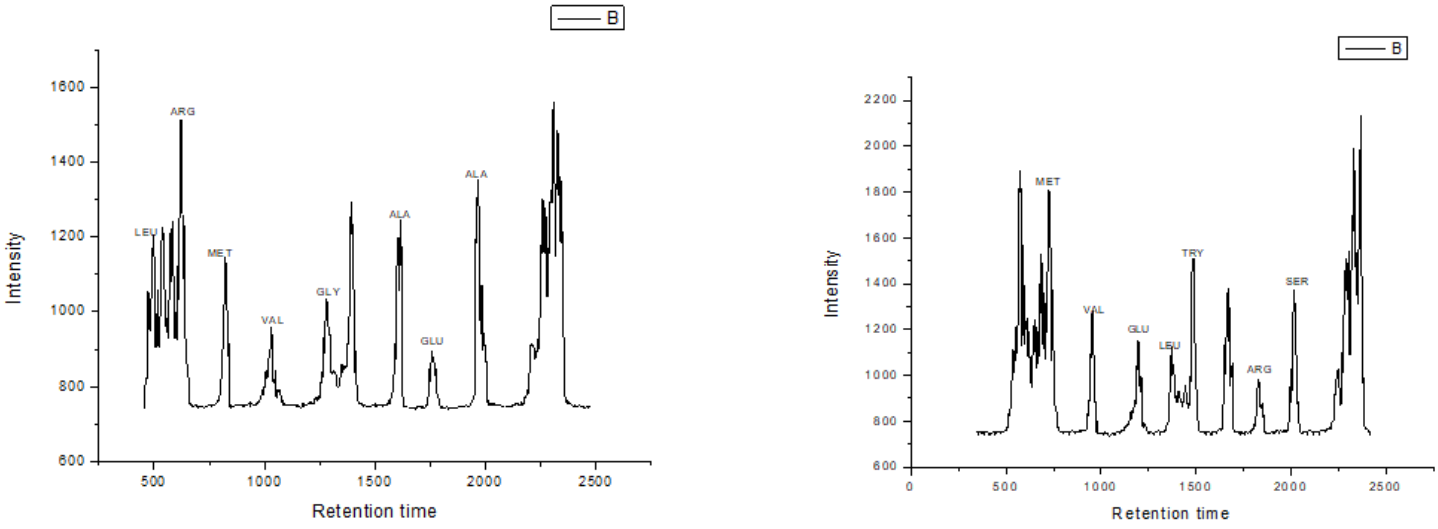


Figure 8

Effect of bead reusability on glutamic acid production from cowpea waste

NB: Corn husk matrix (acid and alkaline treated cowpea waste)



a b

Figure 9

Detection and identification of glutamic acid in the culture broth of *Pseudochrobactrum saccharolyticum*.

a- L-glumatic acid extracellularly produced by *Pseudochrobactrum saccharolyticum* using acid treated cowpea waste

b- L-glumatic acid extracellularly produced by *Pseudochrobactrum saccharolyticum* using alkaline treated cowpea waste