MICROBIOLOGY ARCHIVES
Original Research Article | ISSN: 3041-590X
Journal homepage: https://microjournal.researchfloor.org/

Production and Characterization of Urease from *Aspergillus niger* Using Cassava Peels as Carbon Source Under Solid-State Fermentation

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ARTICLE INFO

Citation: Emochone Roy Yohanna, Yusuf Abayomi Showemimo, Gbadeyan Femi Ayo, Moses Adondua Abah, Silas Verwiyeh Tatah, Sunday Betini John, Micheal Oladosu Abimbola, Okpanachi Nuhu Oyibo, Chinedu Victoria Amarachi, Chioma Stellamaris Enunwoke, Brighotaye Cheery Vaekosen, Amina Jafaru Ocheinehi, Akinyanju John Babajide, Captain Tamunotonye Blessing, Kelechi Asogwa, Sunday Ameh, Nkechi Asogwa, Taiwo Awojulu and Joseph Ezeani (2025). Production and Characterization of Urease from *Aspergillus niger* Using Cassava Peels as Carbon Source Under Solid-State Fermentation. *Microbiology Archives, an International Journal.*
DOI: https://doi.org/10.51470/MA.2025.7.2.40

Received 10 June 2025
Revised 15 July 2025
Accepted 28 August 2025
Available Online September 02 2025

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ABSTRACT

Urease (urea amidohydrolase; EC 3.5.1.5) is an enzyme that facilitates the hydrolysis of urea, primarily synthesized by microorganisms, including algae, fungi, and bacteria. Animals release urea into the soil. Consequently, soil exhibits significant urease activity. Urea in the soil is continuously utilized by microorganisms, particularly bacteria, which decompose it into ammonia and carbon dioxide. Ammonium is frequently employed alongside urea fertilizer to enhance its hydrolysis in the soil. Urease-producing bacteria play important roles in human pathogenicity, biocementation, and soil fertilization, contributing to conditions such as urinary stones, pyelonephritis, and gastric ulceration. This study aimed to produce urease from soil samples, utilizing cost-effective sources and minimizing expenses. It considered the optimization of various culture conditions, including fermentation effects, protein determination, pH, temperature, substrate concentration, and kinetic parameters. Protein determination was employed for the partial purification and assessment of protein levels. Aspergillus niger was cultivated on media supplemented with chloramphenicol to suppress bacterial proliferation. The fungi exhibiting the highest growth were subcultured to establish a pure culture. These fungi underwent urease screening utilizing Christensen's urea agar, where identification was based on the pink coloration resulting from urease degradation, subsequently employed for

fermentation. Solid-state fermentation utilized cassava peel powder as the exclusive carbon source. The fermentation period lasted eight days. Day five exhibited the highest production, characterized by an enzyme activity of 1.47 µM. The influence of pH and temperature on the crude enzyme indicated that the optimal pH and temperature for enzyme activity were 7 and 35°C, respectively, with kinetic parameters (Vmax and Km) recorded at 6.173 and 5.531, respectively. This research investigates a novel and economical method for enzyme production by employing cassava peels, an agro-industrial byproduct, as a carbon source for urease synthesis. The study identified cassava as a potential alternative income source for cassava-producing communities, while also addressing challenges related to food-processing waste management. This study advances green biotechnology, circular economy practices, and the creation of cost-effective, environmentally friendly enzyme production systems that support sustainable development goals.

Keywords: *Urease, Cassava peel, Fungi, Soil, Concentration, Fermentation, and Hydrolysis.*

Introduction

A key component of contemporary manufacturing processes, enzyme biotechnology provides environmentally benign, efficient, and long-term alternatives to chemical catalysts [1]. Because of its numerous uses in biotechnology, agriculture, medicine, and environmental management, urease (urea amidohydrolase, EC 3.5.1.5) has garnered a lot of interest among the several enzymes with industrial importance [2]. An essential step in nitrogen cycling, ureease catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide [3]. This enzyme can be found in many different forms, including plants, bacteria, and fungi. Because of their fast growth rates, simplicity of cultivation, and high enzyme yields, microbial sources are often chosen for industrial applications [4]. A number of fungus, notably *Aspergillus niger,* have shown promise as urease producers due to their adaptability to different substrates and their capacity to secrete extracellular enzymes in a controlled fermentation environment [5]. We need to find sustainable and cost-effective ways to produce urease because its demand is growing in so many different industries [6]. It is economically tough to produce traditional urease on a big scale since it generally uses costly synthetic medium. For this reason, scientists are looking to agro-industrial wastes as a potential substitute for traditional substrates [7]. As a plentiful agricultural waste product in many tropical nations, cassava peels provide one such potential substrate. The world's top producer of cassava, scientifically known as Manihot esculenta, is Nigeria [8]. This staple crop is grown across sub-Saharan Africa, Asia, and Latin America. Many people throw away the large amounts of peels that are produced when processing cassava roots for food and industrial starch, which contributes to environmental degradation [9]. In addition to lowering manufacturing costs and supporting circular bioeconomy projects, valuing these peels as a carbon source for enzyme production tackles waste management concerns [10].

When used to agro-wastes as substrates, solid-state fermentation (SSF) has emerged as the gold standard for producing microbial enzymes [11]. By simulating the conditions in which many filamentous fungi thrive, SSF is able to produce more extracellular enzymes with less water activity than submerged fermentation [12]. The method yields concentrated enzyme products with little downstream processing, uses less energy, and is cheap [13]. The well-studied filamentous fungus *Aspergillus niger* is ideal for SSF because it can penetrate solid substrates, can withstand stress, and produces a variety of extracellular enzymes [14]. So, a promising strategy for economically viable urease production is to use cassava peels as a carbon source in conjunction with *A. niger* in an SSF environment. Finding effective microbial strains requires first isolating urease-producing fungi from their natural habitats, such as soil and agro-waste dumpsites [15]. To select organisms with high enzymatic activity and adaptation to low-cost substrates, screening for powerful isolates is necessary [16].

Optimizing substrate composition, moisture content, temperature, and pH for urease synthesis after isolation further increases enzyme yield [17]. Biochemical characteristics including optimal pH, temperature stability, kinetic parameters, and susceptibility to activators or inhibitors can be ascertained by characterizing the generated enzyme. This type of analysis sheds light on whether or not the enzyme is appropriate for use in certain biomedical or industrial contexts [18].

The manufacturing of ureease is particularly noteworthy due to its numerous applications. Fertilizers based on urea can have their nitrogen losses reduced and soil fertility managed with the help of urease [19]. The efficiency of nitrogen utilization and crop yield can be enhanced through the controlled application of urease, which minimizes volatilization losses [20]. *Helicobacter pylori* is a bacteria associated with gastric cancer and peptic ulcers; urease is useful in the diagnosis of these conditions. The quick urease test and other urease activity-based diagnostic tests depend on the detection of microbial urease [21]. Urease is also used in biosensors that measure the concentration of urea in various bodily fluids; these biosensors are an integral part of the treatment plan for patients suffering from renal failure. Among urease's many uses in environmental bioremediation is the purification of urea-and nitrogenous compound-containing industrial effluent [22]. For soil stability and fracture healing in construction materials, biocementation and bioconsolidation technologies have investigated urease-induced calcium carbonate precipitation. It is crucial to find sustainable and cost-effective ways to produce urease because of its many uses [23].

Cassava peels offer a distinct benefit as a substrate for enzyme production because of their nutritional makeup. They facilitate microbial metabolism and growth due to their abundance of starch, cellulose, hemicellulose, and small proteins [24]. Fungi are able to easily digest the simple sugars found in cassava peels, which increases enzyme secretion. Nevertheless, microbial processing is required to detoxify and valorize the peels due to the presence of anti-nutritional substances including cyanogenic glycosides [25]. In addition to making a contribution to enzyme biotechnology, the work tackles the twin problems of agricultural waste management and environmental sustainability by producing urease from cassava peels. Using *Aspergillus niger* as the microbe is a calculated move [26, 27, 28]. Regulatory agencies have classed this fungus as generally regarded as safe (GRAS), and it is extensively employed in industrial biotechnology [29, 30, 31]. It is able to adapt to conditions with minimal nutrients, produces a diverse spectrum of extracellular enzymes, and has vigorous fermentation capabilities. Its viability for urease production in this context is further shown by its established capacity to grow on substrates obtained from cassava [32].

The objective of the work was to use solid-state fermentation with cassava peels as a carbon source to identify, generate, and characterize urease from *Aspergillus niger*.

There are multiple reasons why this study is important. It begins by looking at a sustainable, low-cost method of producing urease from the widely available agro-waste. Secondly, it helps put the environmentally harmful and underused cassava peels to better use. Thirdly, important information regarding the enzyme's possible uses in farming, healthcare, and industry will be revealed by its biochemical characterization.

Materials and Method

Study Area

The study was caried in the Biochemistry Department, Faculty of Biosciences, Federal University Wukari, Taraba State, Nigeria in the year 2023/2024. The research work lasted for 5 months beginning from November 2023 and ending in March, 2023.

Materials Used for the Study

For this study, the following materials were used: Pressure pot, distilled water, gas cylinder, inoculating loop, conical flask, wash bottle, Aluminum foil, masking tape, spectrophotometer, beaker, test tubes, spatula, syringes, Petri plate, Whitman filter paper, measuring cylinder, weighing balance, Pasteur pipette, 250ml Erlenmeyer flask, fungi isolate, cotton wool, incubator, autoclave, pH meter, water bath, and centrifuge.

Reagents Used for the Study

All chemicals/reagents used in the study were analytical grade and were obtained directly from the manufacturers or purchased from local commercial vendor. These chemicals/reagents include: potato dextrose agar, Christensen's urea agar, urea, phosphate buffer (pH 7.0), casein, HCL, NaCl, Trichloroacetic acid, Folin Ciocalteu's phenol reagent, sodium carbonate, KNO_3, $MgSO_4.7H_2O$, K_2HPO_4, $ZnSO_47H_2O$, $FeSO_4.7H_2O$, $MnSO_47H_2O$, Na_2CO_3, NaOH, NaK Tartrate, $CuSO_4.5\ H_2O$ was purchased from reliable source.

Sample Collection

Aseptically, soil sample weighing 5g, obtained from a depth of 5 to 10 centimeters below the surface, was collected in November 2023 from the Federal University Wukari hostel. Serial dilution was prepared with the obtained soil sample before being transferred into the culture media.

Media Preparation

We followed the procedure outlined in [33] to prepare the media. One gram of potato dextrose agar (PDA) and two milliliters of chloramphenicol were measured and added using a weighing balance in order to inhibit bacterial growth. It was then carefully transferred to a 250 ml Erlenmeyer flask, 100 ml of distilled water was added, and mixed thoroughly. The flask was then covered with aluminum foil and packed tightly with cotton wool. After 20 minutes of sterilization in an autoclave set at 121°C, the medium was withdrawn and left to cool. Before inoculating the fungus isolate into the gelling media, it was placed into a Petri plate for culturing.

Isolation of Fungi Species

The target fungus species was isolated using the procedure detailed in [34]. Different fungi species were isolated from soil samples by serially diluting the samples from 10^2- 10^6. Then, they were cultivated at room temperature in Petri dishes that contained potato dextrose agar. After identifying the sample with the most rapid growth, it was tested to confirm the presence of the urease enzyme. The fungi were subsequently extracted.

Morphological Characterization of Fungi Species

The approach provided by [35] was used to conduct molecular characterisation of fungal species. Colonies of Aspergillus species were subcultured and let to develop for four days after identification by macroscopic features such as color, texture, and diameter of colonies in comparison to the atlas.

Screening for Urease-Producing Fungi Species

For the purpose of detecting urease activity, Christensen's urea agar is utilized [36]. After transferring 2.4 grams of urea agar to a 250 mL Erlenmeyer flask, 100 mL of distilled water was added and the mixture was stirred for a few minutes to conduct the urease screening test. The solution was subsequently autoclaved at 121☆c for 20 minutes, and subsequently cooled at 45-55 ◦ c.When the urea agar base had cooled, 5 milliliters of urea solution was added to it. Be careful not to heat the urea agar mixture again after adding the urea. To get a uniform yellow tint, mix the urea with the urea base agar. After pouring the urea base agar into the test tubes, they were tilted to allow the mixture to harden. Following slant solidification, a thick inoculum was streaked over the whole surface of the slant using a pure culture that had been cultured for 18 to 24 hours. Next, every six hours, every twenty-four hours, and daily for as long as six days, the color change was noted. Urease production is marked by a slanting, sometimes butt-extending, brilliant pink (fuchsia) color. A good result for urease is a brilliant pink color; a negative result is a persistent yellow tint.

Production of Urease Under Solid State Fermentation

50 grams of substrate (cassava peels) was placed in 250 ml conical flasks, along with 100 ml of salt solution (expressed in grams per liter) containing the following components: KNO_3 at 2.0 grams, $MgSO_4.7H_2O$ at 0.5 grams, K_2HPO_4 at 1.0 gram, $ZnSO_47H_2O$ at 0.437 grams, $FeSO_47H_2O$ at 1.116 grams, and $MnSO_4.7H_2O$ at 0.203 grams. The pH of the mixture was adjusted to 7.0, and the flasks were autoclaved for 20 minutes at 121 degrees Celsius to ensure sterility. Each flask was sterilized before being injected with 1.0 ml of a spore solution containing 106 spores per ml. Following that, the inoculated flasks were placed in an incubator set to 30 degrees Celsius and incubated for seven days.

Urease Assay

Urease assay was performed as described by [37]. Enzyme extract (0.25µL) was added to 10ml of urea solution (0.4g urea in 25ml of phosphate buffer). 1 ml of the pervious solution was added to each test tube containing 5ml of Nessler's reagent, and incubated at 40 ◦ c for 5 min. They were followed by the addition of 1.0m HCL to terminate the reaction specific time and the absorbance was taken.

Determination of Protein Concentration

Protein concentration was determined using the method outlined in reference [38]. One milliliter of distilled water was added to two milliliters of BSA working standard before being pipetted into five separate test tubes for protein determination. A 1 ml volume of pure water was used as a control in the test tube. Incubation was carried out for 10 minutes after 4.5 ml of Reagent I was introduced.

Following 30 minutes of incubation, 0.5 milliliters of reagent II was introduced. A standard graph was used to plot the absorbance, which was measured at 660 nm. Use the standard graph to make an estimate of the protein concentration in the provided sample.

Effect of Incubation Temperature on Urease Activity

To ascertain the optimum temperature of urease, fungal spores were inoculated into SSF medium in 250mL Erlenmeyer flasks were incubated at 30°C, 35°C, 40°C, 45°C, 50°C, 55°C and 60°C. To assess the influence of temperature, five test tubes were employed in which 1ml of culture supernatant, along with 5ml of urea solution were added to the various flasks and the mixture was incubated for 10 minutes in a beaker containing water and the temperature was maintained using a water bath. The optimal temperature was then determined [39].

Effect pH on Urease Activity

The influence of starting media pH on urease synthesis was adjusted by varying the pH range of the SSF medium [40]. 2g of casein (substrate) was dissolved in 100ml of phosphate buffer, and 20ml of the phosphate buffer solution was transferred to five beakers with pH levels ranging from 6,7,8,9 and 10. Drops of NaOH and HCL acid were used to modify the pH, and 1ml of culture supernatant was added to each pH variation. After incubating the mixture for 10 minutes at 40°C in a water bath, 6 m of trichloroacetic acid (TCA) was added to stop the reaction, and the liquid was filtered through Whitman filter paper and placed under icy water. The filtrate was then treated with 3ml of Na_2SO_4 solution and 1ml of Folin-Ciocalteau reagent for 10 minutes to generate a color. Finally, enzyme activity was determined using a spectrophotometer.

Effect of substrate concentration on Urease Activity

The concentration of the substrate was varied to 0.5%- 6%, at an interval of 0.5%. In twelve test tubes, 1ml of phosphate buffer and 1ml of enzyme were introduced into 5ml of various substrate concentrations and incubated at 40◦c for 10 minutes.

Determination of Kinetic Parameters of Urease

The kinetic parameters (Km and Vmax) of urease were determined by the double reciprocal plot. The concentration varied from 1 to 8mg/ml and the initial reaction velocities were be used.

Results

Screening for Urease-Producing Fungi Species

Cultures were inoculated into urea base agar in a test tube to screen for urease production in an aseptic manner. Results from the screening for species of fungi that produce urease were encouraging, with isolates of *Aspergillus niger* demonstrating high levels of urease activity. Figure 1 shows that a positive result for urease was indicated by the production of a pink hue.

Figure 1. A test tube showing pink coloration due to the presence urease

Production of Urease Under Solid-State Fermentation

The screened *Aspergillus niger* was then solid-state fermented in an appropriate medium and watched. It was discovered that the isolates could digest cassava peels and generate urease. Over the course of 24 hours of incubation, urease production was progressively boosted until it reached its optimal level. There was a decline in urease production following the optimal incubation time. Also, as you can see in Table 1 and Figure 2 down below, the results showed that the peak output of urease following an 8-day fermentation occurred on day 5.

Table 1. Urease production under solid state fermentation with varying incubation time

Days of Incubation	Activity (µmol/min-1)
1	0.42
2	0.23
3	0.61
4	0.26
5	1.47
6	0.46
7	0.13
8	0.05

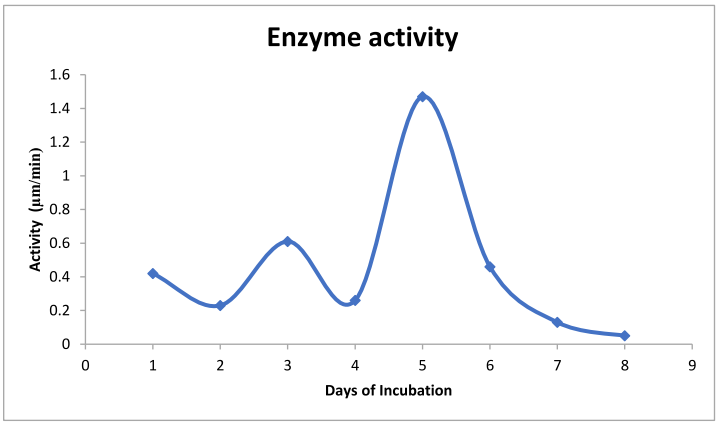

Figure 2. Urease production under solid state fermentation with varying incubation time

Determination of Protein Concentration

Figure 3 and Table 2 show the results of the protein concentration during the incubation time for urease. On the fifth day of incubation, the protein concentration was found to be the greatest. On the other hand, days 1 and 4 had the lowest protein concentrations. After the fourth day of incubation, the protein content rose sharply. On the other hand, following a peak on day 5, protein concentration started to rise again.

Table 2. Protein concentration within the days of incubation

Days of Incubation	Activity(µmolmin-1)
1	0.05
2	0.11
3	0.08
4	0.05
8	0.78
6	0.42
7	0.21
8	0.09

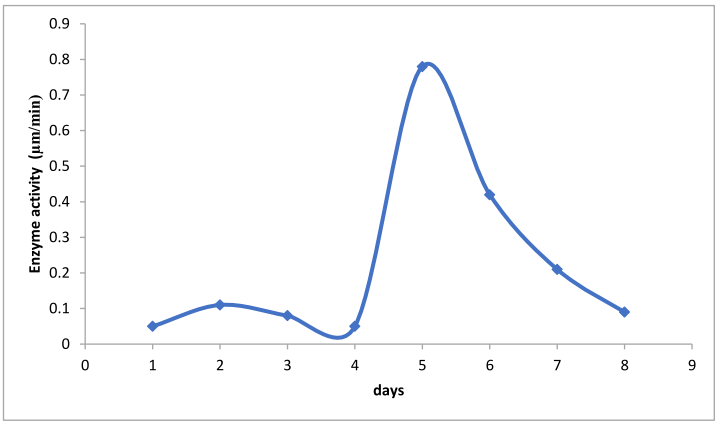

Figure 3. Protein concentration within the days of incubation

Effect of pH on Urease Activity

The impact of different pH levels on urease activity was explored in this study. It was noted that urease activity was 2.536 µmolmin^{-1} at pH 6. Urease activity was 2.935 at a pH of 7. It was noted that urease activity decreased after The urease activity was determined to be 2.493 µmolmin^{-1}, 2.277 µmolmin^{-1}, and 2.220 µmolmin^{-1} at pH 8, 9, and 10 consecutively. According to Table 3 and Figure 4, the highest urease activity of 2.935 µmolmin^{-1} was seen at a pH of 7.

Table 3. Effect of pH on urease activity

pH	Activity (µmolmin^{-1})
6	2.536
7	2.935
8	2.493
9	2.277
10	2.220

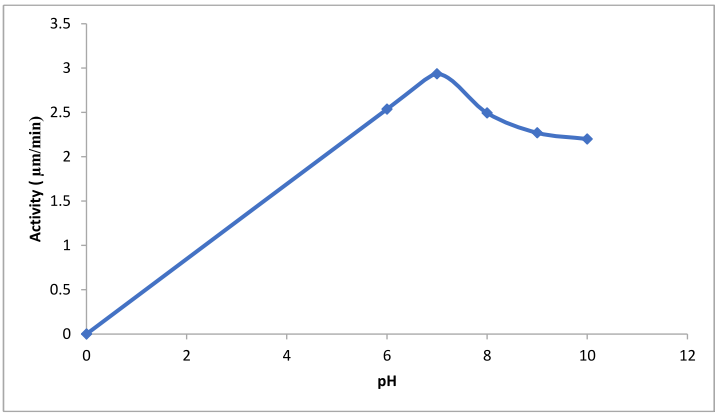

Figure 4. Effect of pH on urease activity

Effect of Temperature on Urease Activity

Table 4 and Figure 5 below show the results of the temperature effect on urease activity. It was discovered that urease activity was most optimal at 350C and least active at 600C.

Table 4. Effect on temperature on urease activity

Temperature (^{0}C)	Activity (µm/min)
30	2.364
35	3.481
40	2.59
45	2.733
50	2.495
55	2.5
60	2.16

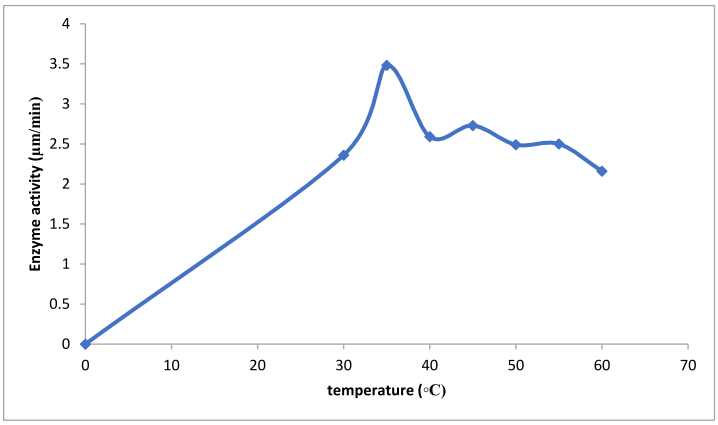

Figure 5. Effect of temperature on urease activity

Effect of Substrate Concentration on Urease Activity

Findings from this study showed that urease's activity fluctuated in response to changes in substrate concentration. As the concentration of the substrate increased, urease activity also increased. Between 2.0 and 5.0 substrate concentrations, urease activity remained relatively constant. According to the results given in Table 5 and Figure 6, the optimal concentration of urease activity is 4%, while the minimum activity was determined to be 1%.

Table 5. Effect of substrate concentration on urease activity

Substrate Concentration (%)	Activity (µm/min)
0.5	1.466
1	1.200
1.5	1.573
2	2.361
2.5	2.533
3	2.535
3.5	2.533
4	2.537
4.5	2.537
5	2.535
5.5	2.411
6	2.535

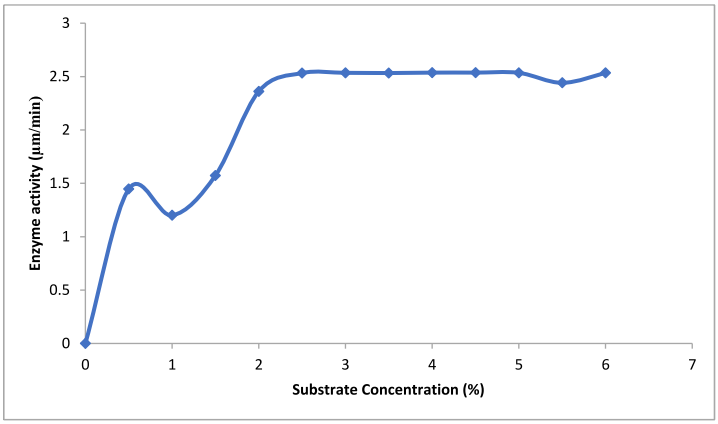

Figure 6. Effect of substrate concentration on urease activity

Determination of Kinetic Parameters of Urease

Urease kinetic parameters were examined in this work. The rate of reaction was determined to be 0.5 at a substrate concentration of 0.423. The rate of reaction was 2. The substrate concentration was 0.682. There was a 0.4 rate of reaction at a substrate concentration of 0.394. The rate of reaction was 2 at a substrate concentration of 0.394. It was typically found that the reaction rate rose linearly with the substrate concentration. Results showed that urease had a Vmax of 6.173 and a Km of 5.531. You can find the urease kinetic data in Table 6 and Figure 7, which are the reciprocal plots (Lineweaver-Burk plots).

Table 6. Kinetic parameters of Urease

1/V	1/S
2	0.682
1	0.833
0.66	0.635
0.5	0.423
0.4	0.394
0.33	0.394
0.28	0.394
0.25	0.394
0.22	0.394
0.2	0.394
0.18	0.414
0.16	0.394

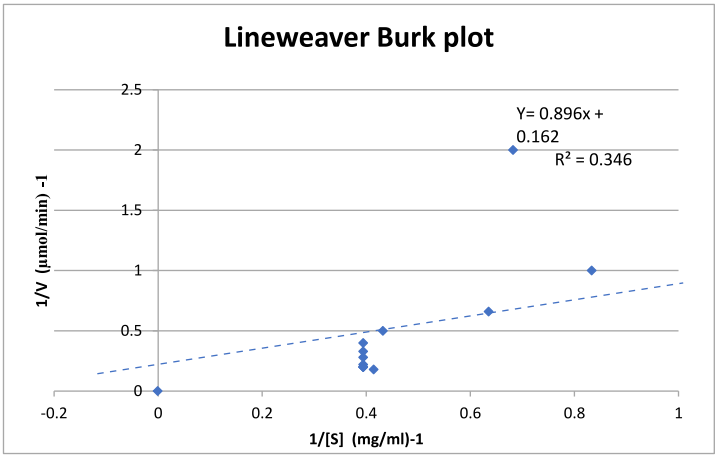

Figure 7. Lineweaver-Burk plot showing the Km and Vmax of urease
Slope = 0.896, intercept = 0.162, Vmax = 6.173 and Km = 5.531

Discussion

All living things rely on enzymes for vital processes. They come from various places of origin [41]. Enzymes are the driving forces behind all intracellular processes, as seen in physiology and biochemistry. Humans, animals, and plants are all susceptible to deterioration and harm caused by changes in enzyme activity [42]. The enzyme industry is one of the most prolific users of enzymes, with applications in a vast array of fields [5, 6]. This includes manufacturing, pharmaceuticals, food and beverage, environmental, healthcare, biotechnology, and many more.

In this investigation, the fungi were inoculated into Christensen's urea agar in a test tube to screen for urease production in an aseptic manner. Figure 1 shows that screened fungal isolates appeared pink in a test tube, indicating that urease was present in the medium.

When the raw fermented product may be utilized directly as an enzyme source, solid state fermentation (SSF) offers great promise for enzyme synthesis [22]. According to [25], SSF has also emerged as a viable alternative for the large-scale synthesis of enzymes and secondary metabolites. The goal of this research was to determine the optimal conditions for solid state fermentation (SSF) of Aspergillus niger in order to maximize urease production. On day 5, the incubation period reached its peak productivity. The range of conceivable variables that affect production, such as temperature, pH, geographical zones, and others, might cause enzymes to have varying peaks in production.

The pH of the solution is one of the factors that influence enzyme activity (26). Being proteins with unique three-dimensional structures, enzyme activity is significantly impacted by the pH of a solution. The optimal pH for the optimal activity of each enzyme has been determined [34].

The optimal pH is critical for substrate binding because it keeps the active site of the enzyme in the correct conformation. Enzyme denaturation can occur if the pH takes a turn for the worse, either because it's excessively acidic or too alkaline (Mallick, 2020). The impact of pH on urease activity was explored in this study. Consistent with previous research, this investigation found that urease was most active at pH 7. According to [6], the highest pH that *Citrullus lanatus* seed extract can withstand is 8.0. According to [7], the optimal pH for Pisum Sativum L seeds grown using Urease is 7.5.

Enzyme activity is also significantly affected by temperature. Enzyme activity decreases with decreasing temperature due to decreased molecular mobility and collisions with substrate [34]. The reaction rate becomes more efficient as the temperature increases, reaching its optimal range at around 37 °C, which is approximately human body temperature. As the temperature rises above the optimal range, it breaks hydrogen and ionic connections, changing the shape and active site of the enzyme and leading to denaturation and inactivation. The current research looked at how urease activity was affected by temperature. Table 4 and Figure 5 illustrate the results of the temperature influence on urease activity, which showed that 350C was the best temperature for urease activity and 600C was the lowest. The results of this study contradict those of [7], who found that 500C was the ideal temperature for producing urease from Citrullus lanatus.

Enzyme activity is known to be directly influenced by substrate concentration. Fewer substrate molecules collide with enzyme active sites at low substrate concentrations, resulting in a sluggish reaction rate [25]. Since more active sites are occupied when the substrate concentration increases, the reaction rate also rises. The enzyme approaches saturation, meaning all of its active sites are full, and the reaction reaches its maximum rate, or Vmax, after some time [26]. More substrate has no longer any effect on activity levels. The results showed that the enzyme's activity altered as the concentration of the substrate changed. With increasing substrate concentration, urease activity rose. From a substrate concentration of 2.0 to 5.0, the urease activity remained relatively constant. According to the results given in Table 5 and Figure 6, the optimal concentration of urease activity is 4%, while the minimum activity was determined to be 1%.

One way to characterize an enzyme's catalytic efficiency is by looking at its kinetic properties [23]. The maximum rate of reaction when the enzyme is completely saturated with substrate is known as Vmax, short for "maximum velocity" [14]. The affinity of an enzyme for its substrate is indicated by its Km (Michaelis constant), which is the concentration of substrate at which the reaction rate is half of Vmax. A low Km value indicates a high affinity [15]. The urease kinetic parameters were examined in this work. We observed a half-reaction rate at a substrate concentration of 0.423. With a substrate concentration of 0.682, the reaction rate was 2. There was a 0.4 rate of reaction at a substrate concentration of 0.394. The rate of reaction was 2 at a substrate concentration of 0.394. As the substrate concentration for the reaction grew, the reaction rate generally increased as well. Results showed that urease had a Vmax of 6.173 and a Km of 5.531. You can find the urease kinetic data in Table 6 and Figure 7, which are the reciprocal plots (Lineweaver-Burk plots).

Recommendation

The use of cassava peels as a carbon source during solid-state fermentation to produce urease from *Aspergillus niger* is an intriguing prospect for a sustainable and economical enzyme manufacturing process. Optimizing fermentation settings, studying enzyme stability, and investigating industrial-scale manufacturing should all be part of future research. Furthermore, it would be advantageous to investigate the possible uses of urease in many domains, including bioremediation, biomineralization, and biosensors. A sustainable and economical solution could be found by increasing output utilizing cassava peels.

Conclusion

This work provides proof that *Aspergillus niger* isolates can be used to manufacture urease. Following the goals and objectives of this study, it is now feasible to use solid-state fermentation to mass-produce crude urease from *Aspergillus niger* with cassava peels as the carbon source. A five-day incubation period, an ideal pH of 7, an ideal temperature of 35 0ºC, and a substrate concentration of 4% were the parameters that allowed this to be accomplished.

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