



Assessment of *Aspergillus niger* and *Bacillus subtilis* Potential in the Biodegradation of Cassava Peels

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ABSTRACT

The processing of cassava for human consumption leads to the generation of large quantities of cassava peel waste. The continuous accumulation of these peels has contributed to environmental pollution, thereby placing significant pressure on waste management systems. This study aimed to assess the biodegradation potentials of *Aspergillus niger* and *Bacillus subtilis*, individually and in combination, using cassava peels as the substrate. Cassava peels were obtained from Sokoto Central Market, Nigeria and subjected to biodegradation using laboratory-maintained isolates. Biodegradation efficiency of the isolates was assessed by determining their percentage weight loss, while changes in pH were monitored over a four-week incubation period. The individual treatments exhibited substantial degradation, with *A. niger* and *B. subtilis* achieving weight losses of 44.8% and 38.7%, respectively, indicating varying degrees of substrate degradation. The co-treatment produced the highest degradation efficiency, with a weight loss of 55.7% and the lowest residual weight when compared with either organism individually. A progressive decline in pH was observed across all treatments throughout the incubation period, indicating that the biodegradation process was accompanied by a progressive shift from near-neutral to acidic conditions in all treatments. These findings demonstrate the capacity of both microorganisms to utilize and degrade cassava peel biomass, with combined culture exhibiting enhanced degradation when compared with the individual treatments. Therefore, further studies incorporating enzymatic, biochemical and molecular analysis are recommended to elucidate the mechanisms responsible for the enhanced combined culture.

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INTRODUCTION

Cassava peel is a major agro-industrial by-products generated by peeling off cassava root. The peel is made almost along the entire phases using cassava for diverse

applications either manual, industrialized or mechanistic utilizations. Processes of cassava utilization such as flour making, starch making, garri production, fufu making and so on, lead to production of peels as end products (Eko et

al., 2019). Cassava peel is one of the by-products that are readily available in countries where cassava is cultivated and processed into food for man. The peel accounts for between 10 to 13 % of tuber by weight, containing of about 5% crude protein and reasonable amount of minerals. Cassava peels possess high starch content that is biodegradable, inexpensive and abundantly available with high starch content (Abel *et al.*, 2021). These by-products are produced in large quantities in Nigeria and are indiscriminately dumped in the environment. About 2.96 million metric tons of cassava peels are generated and discarded annually in Nigeria, for about 10 million metric tonnes of cassava processed for *garri* (a staple Nigerian food) alone (Aro *et al.*, 2010; Mustapha *et al.*, 2019; Onuoha *et al.*, 2020). They serve as co-pollutants with polyethylene plastic wastes and constitute huge environmental nuisance, especially as their organic nature makes them highly degradable by microorganisms usually releasing foul odour to the environment where they are dumped, thus polluting the surface and underground water (Dhanasekaran *et al.*, 2011; Odunfa & Olanbiwoninu, 2012; Onuoha *et al.*, 2020).

Cassava peels are produced at large quantities and could pose a daunting task of environmental pollution with consequential effects on health due to constituents in the cassava fold (Ebah *et al.*, 2021; William *et al.*, 2021). Direct utilization of cassava peel as human food or animal feed is bottleneck because of the availability of anti-nutrients that could elicit toxicity. Particularly presence of substances such as cyanide, oxalate, tannins, phytate, nitrate and so on could be a daunting task in peel management especially among the local community due to low technical know-how (Montagnac *et al.*, 2009). Therefore, searching for more bearable intervention to properly get rid of cassava peel waste is important (Ebah *et al.*, 2021; William *et al.*, 2021).

The extensive processing of cassava for human consumption generates large quantities of cassava peel waste, which has become a serious environmental concern (Kosugi *et al.*, 2009). The continuous accumulation of these peels contributes to environmental pollution, creates unpleasant odors, poses potential health risks, and places significant pressure on existing waste management systems. Current disposal methods for cassava peels are largely inadequate and unsustainable, thereby necessitating the exploration of environmentally friendly waste management alternatives (Aisien & Ikenebombe, 2017). Cassava peels contain toxic substances, notably cyanogenic glucosides such as linamarin and lotaustralin. When these compounds are hydrolyzed by the enzyme linamarase, hydrogen cyanide a highly poisonous compound is released (Zekarias *et al.*, 2019). Exposure to cyanide can result in symptoms such as dizziness, diarrhea, vomiting, nausea, weakness, paralysis, and in severe cases, death (Williams *et al.*,

2021). Due to this toxicity, cassava peels are poorly utilized by animals, further contributing to their accumulation and environmental burden. In addition to cyanogenic compounds, cassava peels also contain several anti-nutritional factors, including phytates, oxalates, trypsin inhibitors, nitrates, and tannins. Chronic exposure to low levels of anti-nutrients has been associated with impaired mineral absorption and long-term health complications (Montagnac *et al.*, 2009; Zekarias *et al.*, 2019). Oxalates reduce the bioavailability of calcium and magnesium, while also interfering with the absorption of iron, copper, and zinc. Phytates negatively affect the absorption of calcium, iron, zinc, magnesium, and molybdenum, whereas trypsin inhibitors interfere with protein digestion by inhibiting the activity of trypsin (Montagnac *et al.*, 2009). Cassava processing generates substantial quantities of peel by-products, which when discarded indiscriminately, can contribute to environmental pollution and pose potential public health risks. Despite their high carbohydrates contents and considerable potential as a low-cost for value added products such as bioethanol, cassava peels remain largely underutilized in many processing communities. The inefficient management of these by-products not only contributes to environmental degradation but also represents a missed opportunity for sustainable bioenergy production and waste valorisation (Aisien & Ikenebombe, 2017).

Biodegradation is the process of breaking or degrading down organic matter into simpler substances by the action of microorganisms like bacteria, algae and fungi (Urbanek *et al.*, 2018). These microorganisms use organic materials as source of energy and nutrients and the process they breakdown complex molecules into simpler ones depending on the type of substance and environmental conditions (Temperature, moisture and oxygen availability) (Sangale *et al.*, 2012). There are certain microbes (bacteria and fungi) that have great potentials in the degradation of agricultural waste. As saprophytes, these microbes have inherent ability to degrade organic substances. Similarly, there are some microbes that are known for their ability to degrade organic based polymers and their by-products such as plastics (Sangale *et al.*, 2012). *Aspergillus niger* is a ubiquitous, diverse habitat inhabiting organisms that grows in dead leaves, grains, composts and soils, as well as other organic materials. *A. niger* is a principal actor that allows its enzyme system utilized for fermentation of substrates at the end giving rise to useful products applicable to disparate human endeavors (Aisien & Ikenebombe, 2017). Similarly, another microbe that could potentially be used to degrade cassava is *Bacillus subtilis*. *B. subtilis* is a well-studied Gram-positive soil bacterium, which undergoes two interesting developmental processes: morphological differentiation into spores (Piggot & Losick, 2001) and physiological differentiation into a state of competence for genetic transformation

(Berka *et al.*, 2002). Furthermore, *Bacillus subtilis* and closely related *Bacillus* species are non-pathogenic, free of endotoxins and well known with respect to fermentation technology, which makes them important cell factories for industrial enzymes, fine biochemicals, antibiotics, and insecticides (Bron *et al.*, 1999; van Dijk *et al.*, 2001).

Aspergillus niger and *Bacillus subtilis* are microorganisms extensively reported for their metabolic versatility and their ability to secrete a wide range of enzymes, including cellulases, amylases, hemicellulases, and proteases, which are crucial for the breakdown of complex organic and lignocellulosic substrates (Pandey *et al.*, 2000; Schallmeyer *et al.*, 2004; Garba *et al.*, 2025). This research will contribute to existing knowledge on microbial-mediated biodegradation processes. By comparatively evaluating *Aspergillus niger* and *Bacillus subtilis*, the study will provide insights into their enzymatic performance, substrate utilization efficiency, and tolerance. These findings will be valuable for optimizing biodegradation and fermentation processes using agricultural waste. Therefore, the study has the potential to address critical environmental challenges, advance scientific understanding of biodegradation and contribute to sustainable development through the effective utilization of cassava peel waste using *Aspergillus niger* and *Bacillus subtilis*. Therefore, this research was aimed to assess the potentials of *Aspergillus niger* and *Bacillus subtilis* in the biodegradation of cassava peels.

MATERIALS AND METHODS

Sample Collection Area

The samples were collected within Sokoto metropolis, Sokoto State, north-western Nigeria, which lies along the border with the Republic of Niger. The region is part of the Sahelian belt, characterized by a predominantly dry, arid climate. The city is located near the confluence of the Sokoto and Rima Rivers, vital for the region's irrigation and water supply needs (Abubakar, 2014). The state lies at an average elevation of approximately 325m above sea level and is characterized by a generally flat to gently undulating topography, with isolated rocky outcrops occurring in some areas (Ahmed *et al.*, 2021). Geographically, Sokoto State is situated between latitudes 13°00' N and 14°00' N and longitudes 4°00' E and 6°00' E, placing it within the semi-arid belt of northern Nigeria. Sokoto State experiences a hot semi-arid climate, classified as BSh according to the Köppen climate classification system. The climate is characterized by high temperatures throughout most of the year, low and erratic annual rainfall, and a prolonged dry season, which is often influenced by dry north-easterly harmattan winds. These climatic and physiographic conditions significantly influence the vegetation pattern, agricultural practices, and environmental processes within the state. The region has long dry seasons from October to May, with

temperatures often reaching over 40°C, particularly between March and June. The rainy season runs from June to September. Annual rainfall is limited, averaging about 600 mm per year, with the most rainfall occurring in August (Abdullahi, 2020).

Collection and Processing of Sample

Cassava tubers were purchased from Central Market, Sokoto, Nigeria, and transported to the Mycology Laboratory, Usmanu Danfodiyo University, Sokoto Nigeria. The tubers were authenticated by a taxonomist in the Department of Botany. The tubers were processed under aseptic conditions to prevent contamination. The cassava tubers were manually peeled using sterile knives to obtain cassava peels. Afterwards, the biomass was pre-washed with running tap water to remove every form of attached impurities followed by rinsing with distilled water (Braide *et al.*, 2016). Then the peels were sliced into smaller pieces and dried in an oven (model no: DHG-9101) for 24 hours to reduce the moisture content (Braide *et al.*, 2016).

Test Organisms

The test organisms used were *Aspergillus niger* and *B. subtilis*. The isolates were obtained from the stock culture collection of Mycology laboratory in the Department of Plant Science and Microbiology Laboratory in the Department of Microbiology respectively Usmanu Danfodiyo University Sokoto.

Media Preparation

Mineral Salt Medium (MSM)

Mineral Salt Medium (MSM) was prepared by dissolving 0.4g potassium dihydrogen phosphate (KH_2PO_4) 0.1 g, sodium chloride (NaCl), 0.002g, calcium chloride dehydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 0.2g, ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$), 0.02g, magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), and 0.001 g Iron (II) sulphate (FeSO_4) in 1000 mL of distilled water. The solution was stirred until complete dissolution, and the pH was adjusted to neutral using Hydrochloric acid (HCl). The medium was sterilized by autoclaving at 121 °C for 15 min and allowed to cool to room temperature before use (Atlas, 2010; APHA, 2012; Cappuccino & Welsh, 2017).

Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) was prepared according to the manufacturer's instructions by dissolving 39g of PDA powder in 1000mL of distilled water. Streptomycin (1g) was added to inhibit bacterial growth. The medium was mixed thoroughly, heated to ensure complete dissolution, and sterilized by autoclaving at 121°C for 15 minutes (APHA, 2012).

Nutrient Agar (NA)

Nutrient agar was prepared according to standard laboratory procedures by dissolving 28g of nutrient agar powder in 1000mL of distilled water. The medium was heated with continuous stirring to ensure complete dissolution and subsequently sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to approximately 45°C and aseptically poured into sterile Petri dishes. The solidified nutrient agar plates were used for the cultivation and maintenance of *Bacillus subtilis* (Atlas, 2010; Cappuccino & Welsh, 2017).

Preparation of Microbial Suspensions

Aspergillus niger

Spore suspensions were prepared according to the method adopted by Singh & Nain (2014). Briefly, 10mL of sterile distilled water containing two drops of 0.1% Tween 80 was added to seven-day-old sporulated cultures. The spores were dislodged using a sterile wire loop under aseptic conditions and homogenized by thorough mixing. The spore concentration was determined with a Neubauer haemocytometer and adjusted to 2.0×10^5 spores mL⁻¹.

Bacillus subtilis

A pure culture of *Bacillus subtilis* was subcultured onto nutrient agar plates and incubated at 37°C for 24h. Following incubation, several well-isolated colonies were aseptically picked using a sterile inoculating loop and suspended in sterile physiological saline (0.85% NaCl). The suspension was vortexed thoroughly to obtain a uniform bacterial suspension. The turbidity of the suspension was adjusted to match a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU mL (CLSI, 2023).

Confirmation of Microbial Isolates

Confirmation of *Aspergillus niger*

The stock for *A. niger* was inoculated onto potato dextrose agar (PDA) plates and was incubated at 28°C for 5 days. The isolates were studied macroscopically for colony morphology and microscopically using Lactophenol cotton blue staining, identification was confirmed based on standard taxonomic keys (Deacon, 2006; Cappuccino & Welsh, 2017).

Confirmation of *Bacillus subtilis*

Isolate was streak onto nutrient agar (NA) plates using a sterile wire loop. Plates were incubated at 37°C for 24 hours. The Isolate was identified using the following tests; Gram staining, endospore staining, catalase test, oxidase test and motility test Identification based on standard morphological and biochemical characteristics (Cheesbrough, 2006).

Biodegradation Assay

10ml of *Aspergillus niger* and *B. subtilis* suspensions were inoculated into 500ml conical flasks containing 300ml of the formulated MSM media and 20g of cassava peels using wire loop after been flamed and was labelled as T1 (*Aspergillus niger*) and T2 (*B. subtilis*), respectively. 5ml each of *Aspergillus niger* and *Bacillus subtilis* suspensions were inoculated into another 500ml conical flask containing 300ml of the formulated media and 20g of cassava peels labelled as T3. Then, another 500 ml conical flask containing 300ml of the formulated media and 20g of cassava peels devoid of any microbial inoculate was labelled T4, where it served as the control experiment. The flasks were aerobically incubated in an incubator at 35°C for 28 days. All tests were performed in triplicates for each treatment (T1, T2, T3, T4). The tests were withdrawn at an interval of 7 days (day 0,7,14,21,28) after incubation. A pH meter was used to measure the pH in order to monitor microbial activity (Hadad *et al.*, 2005; Kyaw *et al.*, 2012). Weight changes of each treatment were measured gravimetrically using a microbalance, and the percentage weight loss was determined using the following formula:

$$\text{Weight loss (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Initial weight: 20g

Statistical Analysis

All data obtained from the experiment were recorded in percentage, mean $\pm$ standard deviation and presented in scientific tables.

RESULTS AND DISCUSSION

Confirmation and Characterization of Microbial Isolates

The microbial isolates used in this study (*Aspergillus niger* and *Bacillus subtilis*) were confirmed based on their macroscopic, microscopic, and biochemical characteristics. The results of their identification are presented in Table 1.

Table 1: Macroscopic and Microscopic Characteristics of Microbial Isolates Obtained

Code	Macroscopic Characteristics	Microscopic and/or Biochemical Characteristics	Confirmed Organism
F ₁	Colonies initially white, later turning black with maturation. Surface shows pale yellow margins with radial fissures.	Septate hyphae with radiating conidial heads becoming columnar at maturity. Conidia smooth, globose, and darkly pigmented.	<i>Aspergillus niger</i>
B ₁	Cream to off-white colonies, smooth, moist, and slightly opaque.	Gram-positive, rod-shaped cells occurring singly and in short chains. motile, catalase positive, endospore-forming.	<i>Bacillus subtilis</i>

Biodegradation Assay

The results of the biodegradation assay are presented in Table 2. A progressive reduction in the weight of cassava peel samples was observed across all treatments throughout the four-week experimental period. The monoculture treatments, T1 and T2, recorded percentage weight losses of 44.8% and 38.7%, respectively, indicating

varying degrees of substrate degradation. The co-culture treatment (T3), consisting of *Aspergillus niger* and *Bacillus subtilis*, recorded the highest percentage weight loss (55.7%) and the lowest residual weight at the end of the experiment. In contrast, the control treatment (T4) exhibited the least reduction in weight, with a percentage weight loss of 19.7%.

Table 2: Weight of Degraded Cassava Peels Samples Collected within Sokoto Metropolis

S/N	Isolates	Weeks (mean±SD) (mg/ml)			
		1 st	2 nd	3 rd	4 th
1	T1	16.60±0.10 ^a	14.60±0.50 ^b	14.07±0.15 ^b	11.03±0.15 ^c
2	T2	18.13±0.21 ^a	16.43±0.21 ^b	14.53±0.42 ^c	12.27±0.15 ^d
3	T3	14.53±0.15 ^a	12.07±0.21 ^b	10.10±0.20 ^c	08.87±0.06 ^d
4	T4	19.03±0.06 ^a	18.57±0.25 ^b	17.30±0.36 ^c	16.07±0.15 ^d

Keys: T1 = *Aspergillus niger*, T2 = *Bacillus subtilis*, T3 = *A. niger* + *B. subtilis*, T4 = Control. Means followed by the same superscript letter within a column are not significantly different at $P \leq 0.05$

Changes in pH During Biodegradation

The pH values recorded during the biodegradation process are presented in Table 3. A gradual decline in pH was observed across all treatments over the four-week period. The co-culture treatment (T3) recorded the greatest decrease in pH, reaching a final value of 3.10 ± 0.10 by the

fourth week. This was followed by T1 (3.87 ± 0.51), T2 (4.70 ± 0.10), and the control treatment, T4 (4.90 ± 0.26). The results indicate that the biodegradation process was accompanied by a progressive shift from near-neutral to acidic conditions in all treatments throughout the experimental period.

Table 3: pH of Degraded Cassava Peels Samples for Four Weeks Interval

S/N	Isolates	Weeks (mean±SD)				
		0	1 st	2 nd	3 rd	4 th
1	T1	5.7±0.57 ^a	5.3±0.35 ^a	3.4±0.55 ^c	4.0±0.76 ^b	3.87±0.51 ^b
2	T2	5.6±0.44 ^a	4.9±0.10 ^b	3.8±0.30 ^d	3.3±0.97 ^e	4.7±0.10 ^c
3	T3	5.7±0.26 ^a	4.4±0.47 ^b	3.6±0.25 ^c	3.5±0.59 ^c	3.1±0.10 ^c
4	T4	5.9±0.10 ^a	5.4±0.31 ^a	3.7±0.36 ^c	4.7±0.69 ^b	4.9±0.26 ^b

Keys: T1- *Aspergillus niger*, T2- *Bacillus subtilis*, T3-Co culture & T4- Control. Means with the same superscript are note significantly different at 5% level of significance

Discussion

Table 1 presents the macroscopic, microscopic and biochemical characteristics used to confirm the test organisms as *Aspergillus niger* and *Bacillus subtilis*. Isolate F₁ showed colonies that were initially white but gradually became black with maturity, with septate hyphae, radiating conidial heads and dark, globose conidia. These characteristics are consistent with the classical morphological features of *A. niger* described in standard fungal identification literature by Deacon (2006)

and Cappuccino & Welsh (2017). Similarly, isolate B₁ produced cream to off-white, smooth and moist colonies and exhibited Gram-positive, rod-shaped cells, motility, catalase positivity and endospore formation, characteristics consistent with *B. subtilis*, as noted by Cheesbrough (2006). The confirmation of these organisms was important because their established extracellular enzyme-producing abilities provide a biological basis for their potential to degrade complex agricultural residues. *A. niger* is particularly recognized for producing hydrolytic

enzymes involved in the degradation of plant cell-wall components, including cellulases, hemicellulases and pectin-degrading enzymes (de Vries & Visser, 2001). Similarly, Schallmey *et al.* (2004) and van Dijk & Hecker (2001) stated that *B. subtilis* is widely recognized for extracellular enzyme production and has important applications in industrial biotechnology because of its capacity to secrete hydrolytic enzymes. Thus, the characteristics reported in Table 1 confirm the suitability of the two organisms for subsequent cassava-peel biodegradation experiments.

Table 2 demonstrates a progressive reduction in the weight of cassava peels throughout the four-week experimental period, indicating that both microorganisms were capable of utilizing and transforming the cassava-peel substrate. The *A. niger* treatment (T1) recorded a reduction from 16.60 ± 0.10 in the first week to 11.03 ± 0.15 in the fourth week, while *B. subtilis* (T2) decreased from 18.13 ± 0.21 to 12.27 ± 0.15 . The co-culture treatment (T3) exhibited the greatest reduction, reaching 8.87 ± 0.06 by the fourth week, whereas the control (T4) retained the highest residual weight of 16.07 ± 0.15 . This indicates that the microorganisms enhanced substrate degradation compared with the uninoculated treatment. The superior performance of T3 is particularly important because the reported overall weight loss was 55.7%, compared with 44.8% for *A. niger* and 38.7% for *B. subtilis*. The greater degradation obtained with the co-culture may be associated with complementary enzymatic activities of the two microorganisms. Cassava peel contains readily degradable carbohydrates as well as structural polysaccharides such as cellulose and hemicellulose, which require different hydrolytic activities for efficient breakdown (Montagnac *et al.*, 2009; Tonukari *et al.*, 2023). Aderemi & Nworgu (2007) demonstrated the potential of *A. niger* to effectively biodegrade cassava peels, resulting in reductions in fibre components and improvement in some nutritional characteristics. Their findings support the substantial-reducing capacity observed for *A. niger* in the present study. Similarly, Iyayi & Losel (2001) and Lawal & Iyayi (2014) documented that microbial treatment of cassava-derived substrates facilitates the breakdown of complex carbohydrates and improve their potential for subsequent biotechnological utilization. Although, the experimental conditions and assessment parameters differed among these studies, their findings generally agree with the present observation that microbial treatment facilitates the transformation of cassava-derived substrates. The higher performance of the co-culture in the present study may therefore reflect complementary substrate utilization, where fungal and bacterial enzyme systems collectively improved access to different components of the cassava peel.

The relatively low weight reduction observed in T4 is also important because it indicates that some mass loss

occurred in the absence of deliberate microbial inoculation. The control declined from 19.03 ± 0.06 in the first week to 16.07 ± 0.15 in the fourth week. Such reduction may partly be associated with physicochemical processes, including dissolution or loss of soluble components from the cassava peel during incubation. Nevertheless, the substantially greater reduction recorded in T1, T2 and particularly T3 indicates that microbial activity contributed considerably to the overall substrate loss. The finding agrees with studies by Aderemi & Nworgu 2007 and Aisien & Ikenebomeh 2017, which shows that cassava peels can serve as substrates for microbial degradation and enzyme production. Therefore, the result obtained in Table 2 supports the potential application of *A. niger* and *B. subtilis*, particularly in combination, for biological management and valorization of cassava-peel waste.

Table 3 shows that the biodegradation process was accompanied by changes in pH in all treatments. The initial pH values were relatively similar, ranging from 5.6 to 5.9, but generally decreased during incubation. At the end of the fourth week, T1, T2, T3 and T4 recorded pH values of 3.87 ± 0.51 , 4.70 ± 0.10 , 3.10 ± 0.10 and 4.90 ± 0.26 , respectively. The greatest acidification occurred in T3, which also recorded the greatest weight loss in Table 2. This relationship suggests that increased microbial metabolism was associated with the production or accumulation of acidic metabolites during degradation. The marked acidification observed in the *A. niger*-containing treatments is biologically plausible because *A. niger* is well known for producing organic acids, particularly citric acid, during fermentation of carbohydrate-rich substrates. Previous study by Okareh & Enesi (2019) have demonstrated the use of *A. niger* fermentation of cassava peels for organic-acid production, further supporting the ability of this organism to modify the chemical environment during cassava-peel fermentation. The decrease in pH may therefore reflect the conversion of cassava-peel carbohydrates into organic acids and other metabolic products. The present observation of a decrease from the initial near-neutral pH to 3.87 in T1 and 3.10 in T3 is therefore broadly consistent with the acid-producing characteristics previously reported.

The co-culture treatment had both the greatest substrate weight reduction and the lowest final pH. This suggests that the enhanced biodegradation observed with the combined organisms was accompanied by increased metabolic activity. Similar relationships between microbial degradation and changes in physicochemical conditions have been reported by Pandey *et al.* (2000) and Pérez *et al.* (2002) during the biological conversion of agricultural residues, where substrate hydrolysis and subsequent microbial metabolism result in accumulation of acidic metabolites. Nevertheless, the pH decrease should not be interpreted as a direct quantitative measure of biodegradation because the uninoculated control also

experienced some acidification. The control decreased from 5.9 ± 0.10 to 4.9 ± 0.26 over the experimental period, indicating that physicochemical changes and/or indigenous microbial activity may also have contributed to the pH variation.

The pH pattern observed may also have influenced the degradation process itself. Microbial growth and extracellular enzyme activity are strongly dependent on environmental pH, and excessive acidification can eventually reduce the activity of some microbial enzymes. Therefore, the progressive acidification observed during the 28-day experiment may have contributed to changes in degradation rate over time. The findings emphasize the importance of monitoring pH when optimizing microbial conversion of cassava peel and other lignocellulosic residues. Future studies should investigate pH optimization together with enzyme activities, reducing-sugar production and residual cellulose, hemicellulose and starch to establish the precise relationship between acidification and substrate degradation.

CONCLUSION

This study successfully achieved its aim by demonstrating the biodegradation potential of *Aspergillus niger* and *Bacillus subtilis* on cassava peel biomass. Both microorganisms were capable of degrading cassava peels individually; however, the co-culture system exhibited superior degradative ability, indicating that synergistic interactions between the two microorganisms enhanced substrate utilization and overall biodegradation efficiency.

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