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Isolation and screening of cellulolytic fungi for the pretreatment of lignocellulosic biomass (agro-waste) for biogas production

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Abstract

Agricultural waste (agrowaste), an abundant waste in Nigeria and Africa, is gradually replacing crude oil World wide through its bioconversion into biogas (methane gas) and biofuels (biodiesel) as an alternative source of energy. The only limitation encountered is the need for a pretreatment prior the process of biogas and or biodiesel production due to presence of lignocelluloses in the waste. . This study presents an investigation of the ability of different fungi isolates to degrade the lignocellulosic component of plantain trunk wastes through biological pretreatment. Five fungi isolates were isolated from the decomposing agrowaste (plantain wastes) and were screened for cellulolytic activities on selective CMC agar using the plate screening method. *Aspergillus niger* exhibited the highest cellulolytic activity on the CMC agar at pH 4 with an ICMC value of 1.55 followed by Trichoderma spp with an ICMC value of 1.31, while the reverse was observed for the cellulolytic activity on the CMC agar at pH 5, with T. spp exhibiting the highest ICMC value of 1.45 and *A. niger* with an ICMC value of 1.25. While the other isolated fungi isolates, *Aspergillus flavus, Aspergillus fumigatus*, and *Rhizopus stolonifer*, exhibited no definite cellulolytic activities. The inocula of both A. niger and T. spp were used for the pretreatment of the plantain trunk waste, separately and jointly using the Solid State Fermentation (SSF) technique for a week. The highest reduction in lignin and cellulose content was observed when the waste was pretreated with both fungi isolates (in equal proportions) with a reduction of 28.59% (18.85% to 13.4%) and 43.77% (59.22% to 33.0%), respectively followed by pretreatment with T. spp with 22.44% (18.85 to 14.62%) and 43.77%(59.22 to 39.61%) reductions and *A. niger* with 18.3% (18.85 to 15.4%) and 31.8% (59.22 to 40.38%) reductions, respectively. Thus, co- digestion or pretreatment of waste with two or more fungi, will be most favourable for the biological pretreatment of lignocellulosic waste (agrowaste) prior biogas production.

Keywords: Agrowaste, Pretreatment, Biogas, Fungi isolates, Solid State Fermentation (SSF)

Introduction

Man's over-growing population parallels his need for energy and the straining dependence on the conventional fossil fuels has made the option of/ search for an alternative form of energy popular. The rising cost of crude oil, the environmental concerns of carbon dioxide (CO_2) emission associated with the use of fossil fuels and the alarming depletion of the fossil fuel reserves are also factors that support researches in an alternative renewable energy source (Kumar *et al*., 2009; Dashtban *et al*., 2009; Perera, 2017). Moreover, the global crude oil production is predicted to decline from 25 billion barrels to approximately 5 billion barrels by 2050 (Campbell and Laherrere, 1998). Biogas production can be categorized as one solution for this renewable energy need (Paepatung *et al*., 2009), as it eliminates the biohazardrous emission of carbon dioxide -CO_2 and it utilizes a diverse and readily available source of raw materials. The substrate (raw material) of choice plays a vital role in the productivity and stability of the biogas produced, although the microbes constituted in every substrate differ (Szila´gyi *et al*., 2021). These raw materials include biomass, sewage, manure, industrial waste and municipal waste amongst others.

In most developed countries, biogas production is being widely utilized for heat and power generation, for instance in the United States of America; the biogas produced from sewage is used to power the street lights of a whole city (Rezaiyan and Cheremisinoff, 2005 ; Kabeyi and Olanrewaju, 2022). Africa, with its abundance of biomass such as agro waste, municipal waste, industrial and water waste is

yet to fully exploit the utilization of this process for power generation (Mshandete and Parawira, 2009). Mshandete and Parawira (2009) in their review of the utilization of biogas technology in some sub-Saharan African countries revealed that although a few of these countries currently utilize this technology, more research still needs to be carried out to evaluate the potential and feasibility of various available biomass and the different designs of anaerobic biodigester that can be used for biogas production.

Biogas production involves the anaerobic digestion of selected feedstocks to yield biogas comprising mainly of methane (60%-70%), and other gases; carbon dioxide (20%-30%) and traces of hydrogen sulphide (Yerima, 2001; Li *et al*., 2019), which are impurities. Biogas is a form of Biofuel generated from the anaerobic digestion of organic matter. Purified biogas can be compressed for storage and used for electricity generation, heating, lighting and other energy needs (Tambawal, 2002). Generally, Biofuels give off a higher oxygen levels of 10–45% and very low levels of sulphur emission compared to petroleum-based fuels which have no oxygen levels with high sulphur emission laying emphases on the safety of biofuels (Khan *et al*., 2018). The major objective of biogasification is the conversion of feed stock into more valuable, environmentally friendly intermediate products which can be utilized as chemical fuel and energy production (Rezaiyan and Cheremisinoff, 2005).

Bioconversion is another process that can assist in alternative power generation and waste management. It is the conversion of organic matter (waste) through some biological (microbial) activities into more valuable and usable material or energy source (Rezaiyan and Cheremisinoff, 2005). The process yields more options of alternative forms of energy, such as bioethanol (Biofuel) and biogas. Proper and large scale utilization of the Biogas technology and Bioconversion in Africa will not only take care of her pressing power needs but also pave way for proper waste management and the reduction of diseases associated with poor waste handling. Nigeria, with approximately 140 million people, a growth rate of 2.38 and the largest population in Africa (Ogwueleka, 2009), will surely benefit from utilization of these technologies.

Several feedstocks have been utilized for biogas production over time. The conventional use of Cow and Swine dung has been an age-long practice in the rural areas of India and China, respectively. Other feedstock materials such as sewage, water hyacinth, agrowaste, municipal waste and other biodegradable waste have also been extensively utilized in different parts of the World. Of all the utilizable raw materials in bioconversion and biogas production, agricultural waste has proven to be the most sustainable source of biomass that can be utilized (Muthangya *et al*., 2009b; Maurya *et al*., 2015), due to their abundance and energy packed nature. Unfortunately, improper waste management has become an obstacle to the optimal use of the waste materials leading to low utilization(Bala *et al*., 2023).Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin (Yat *et al*., 2008; Tarasov *et al*., 2018). Lignocellulosic, a major form of agromass possesses a complex structure that requires pretreatment to break down and simplify (Bala *et al*., 2023) Lignocellulosic biomass is a renewable organic matter and a major structural component of all plants (Dashtban *et al*., 2009). Sheer enormity of lignocellulosics makes them potential feedstock for biofuel production but, their conversion into fermentable sugars is a major hurdle (Saritha *et a*l., 2012).The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion to fuel. For the conversion of biomass to biofuels, the cellulose and hemicelluloses contained in the biomass must be broken down into their corresponding monomers (sugars), so they can be utilized by microorganisms (Kumar *et al*., 2009). Different biomass are composed of lignocellulose components in different proportions

In order to enhance biogas yield during biogas production, pretreatment of the feedstock is essential. Pretreatment of lignin-containing biomass prior to anaerobic digestion has proven to be an effective method to improve their biodegradability and biogas production from them (Chen et al., 2005, Olatunji *et a*l., 2021). Several pre-treatment methods can be used, they include; physical/mechanical (e.g milling, & grinding), chemical (e.g., alkali, dilute acid & organic solvents), physiochemical, and biological, or a combination of these (Björnsson *et al*., 2005; Mshandete *et al*., 2008). Dar and Tandon (1987) observed an increment of 31-42% microbial digestibility and an almost two-fold increase in biogas yield when alkaline treated plant residue was co-digested with cowdung. Several works have also shown that the pretreatment of agro waste greatly enhances the process of biogas production (Muthangya *et al*., 2009b). Pretreatment of biogas is essential to remove impurities, increase methane content, prevent corrosion, reduce odors, and meet quality standards, enabling efficient and safe utilization of the biogas for various applications (Kovács *et al*., 2022). Moreover, biological pre-treatment is gaining prominence as an ecologically sustainable approach characterized by its low energy requirements, cost-effectiveness in waste

disposal, operation under mild conditions, and limited generation of by-products.(Mira *et al*., 2015)

The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors in the conversion of lignocellulosic biomass to Biofuel (Howard *et al*., 2003; Lee *et al*., 2014). The biomass needs to be treated so that the cellulose will be made accessible for hydrolysis (Kumar *et al*., 2009). Cellulose, which makes up to about 40-50% of plant`s composition is the most abundant organic matter on earth (Omojasola and Jilani 2008; Shafique et al., 2009). Pretreatment of cellulose enclosed within the lignocellulosic material opens the structure and removes secondary interaction between glucose chains (Tang *et al*., 1996; Huang et al., 2022), and the recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria (Baig *et al*., 2004).

Microbial pretreatment of agricultural waste involves the use of microbes, mostly fungi to break down cellulose through the production of the enzyme, cellulase. Fungi are the main cellulase producing microorganism, though some bacteria isolates have been reported to also yield cellulase activity (Immanuel *et al*., 2007). Cellulases are a group of hydrolytic enzymes capable of hydrolyzing cellulose to smaller sugar components like glucose units (Kader *et al*., 1999 ; Behera *et al*., 2017). They have been extensively utilized for extraction of valuable components from plant cells, improvement of nutritional values of animal feed and the preparation of plant protoplasts in genetic research (Mandels, 1985). The cellulase producing ability of various fungi have been studied, but the cellulolytic enzyme system of Trichoderma reesei is the best-studied fungal example (Omojasola and Jilani, 2008), probably because it was one of the first fungi found to exhibit cellulolytic activity (Howard *et al*., 2003).

Microbial cellulases have industrial application in the conversion of cellulose into glucose (Kumar *et al*., 2008). Today cellulase enzymes are industrially produced from genetically mordified stains of both *Aspergillus niger* and Trichoderma species (Omojasola and Jilani, 2008). The enzyme is made up of three main parts; the endo-glucanase, exo-glucanase and cellobiohydrolase (Klyosov, 1990) which are extracellular and inductive in nature (Enari, 1983). The enzymes work synergically in the breakdown of cellulose during pretreatment. Endo- glucanase degrades cellulose into scarred cellulose, while exo-glucanase degrades scarred cellulose into cellubiose and finally cellobiohydrolase degrades cellubiose into glucose (Lee, 2008) which is readily utilized by microbes during bioconversion and biogas production.

Lignocellulosic wastes are usually pretreated with pure industrially produced enzymes (from selected fungi isolates) or by directly spreading cellulolytic fungi on the waste. Solid State Fermentation (SSF) is one of the most economically viable processes used in the bioconversion of lignocellulosic waste (Kanmani *et a*l., 2009). It is an an attractive process that produce fungal microbial enzymes (Chahal *et al*., 1996). One of the most important environmental benefits of biological pretreatment is that it is green process without use of any chemicals and there is no need for chemical recycling and it does not release any hazardous or toxic compounds to the environment (Sindhu *et al*., 2016) and the byproduct produced during the procedure does not inhibit subsequent hydrolysis as well as fermentation.

The aim of the study is to screen for the cellulolytic activities of different fungi isolates isolated from decaying agricultural waste and to use the strain with the best cellulolytic activity to pretreat plantain trunk waste.

Materials and Methods

The research procedure is divided into three major parts. The microbial analysis and cellulase screening assay, and post treatment proximate analysis. All microbial works were carried out in the mycology laboratory of the Botany department, UNILAG.

Microbial Analysis And Cellulolytic Screening Assay

Fungi isolates were isolated directly from decomposing plantain waste in the UV room as described by Booth (1971) by soaking the plantain waste in a sterilant (JIK[NaOCl]: water 60:40 v/v) for about one minute to remove surface contamination, and double rinsed in sterilized water. The rinsed waste was then cut into bits of 0.3cm and plated on PDA media containing chloramphenicol (250mg) to prevent bacterial contamination (Booth, 1971). The plate was incubated at 280C for 2-5 days after which sub-cultures were done to get pure cultures.

Pure isolates were identified morphologically and microscopically(photomicrographs) by mycology experts in the Department of Botany, University of Lagos.

Enzymatic Assay: Cellulolytic Activity Using Plate Screening Method

The isolated fungi were screened for Cellulolytic activity using the Plate Screening method as described by Abu-Bakar *et al.* (2010). Fungi strain were cultured on a Carboxymethyl Cellulose (CMC) selective agar, comprising of 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.2% CMC Sodium (Na) salt (low viscosity), 0.05% KCl, 0.02% Peptone, and 1.7% agar-agar at pH 4 and 5. The agar was poured in triplicates to allow for mean values, and incubated for 6 days at

28°C after inoculation with fungi isolates. The pH of the agar were adjusted with 10% NaOH and dilute HCl.

The Cellulolytic activity was assayed for based on the diameter of zone of hydrolysis surrounding the colony as described by Dále (2007). For observation, culture plates were stained with 0.33% Congo red dye for 15 minutes and then destained by flooding with 1M NaCl solution for 20 minutes. The diameter of the colony before staining and the diameter of the zone of hydrolysis were measured.

Cellulase activity on CMC agar was recorded as the Index of Relative Enzymatic activity (ICMC) as described by Hankin and Anagnostakis (1977), Teather and Wood (1982) and Bradner et al. (1999). The higher the ICMC value the higher the cellulolytic activity.

$$ICMC = \frac{\text{Clear zone (hydrolysis) diameter}}{\text{Colony diameter}}$$

Fungi Inocula Preparations and Pretreatment Of Agrowaste

The inocula of the fungi isolates with the best Cellulolytic activities were prepared on wheat grains using the Mushroom inocula technique as described by Stamets (2000) while the inocula multiplication were prepared using a modified method of Pathmashin *et al.* (2008) and the bag culture method of Stamets and Chilton (1983). A large portion of the inocula was spread on enriched sawdust, containing equal percentages of sawdust and wheat flour, 0.2% $MgSO_4$, 2% $CaCO_3$ and an optimum amount of sterilized water after which it was incubated for eighteen (18) days at 280C ± 20C. The resulting fungal spread was then used for the biological pretreatment of the waste.

Agrowaste Pretreatment

The plantain trunk wastes were collected from the Idi-iroko mass plantain sales site Mushin, Lagos metropolis. The plantain trunks, after detachment from the plantain bunch are usually discarded on the service lane nearby. Investigation from the dealers revealed that the trunk was of no valuable use and so were discarded as waste.

The collected Plantain trunk wastes were chopped into little bits of 6-50mm particle size as a form of physical pretreatment after which biological pretreatment of the waste was carried out using the Solid State Fermentation (SSF) method as described by Muthangya e*t al.* (2009b).

Proximate Analysis

Parameters such as the percentage moisture content, Total solid (TS)/ dry matter, Total volatile solid (VS) were determined by standard methods (APHA, 1995 ; Kechea *et al.*, 2022), cellulose, lignin and hemicellulose contents were determined using methods as described by Updegraff (1969). The Analysis were carried out before and after pretreatment of waste, at the Department of Biochemisrty Idi-Araba, College of Medicine, University o Lagos.

Results

Five pure fungi isolates (Table 1; Plates 2-6) were sub- cultured from the mixed culture (Plate 1). the photomicrographs of the isolates are shown in Plates 7-11.

TABLE 1: List of isolated fungi

PLATE	FUNGI
1	*Aspergillus niger*
2	*Aspergillus flavus*
3	*Aspergillus fumigatus*
4	*Trichoderma species*
5	*Rhizopus stolonifer*

PLATE 1: Mixed culture plate

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Pure culture plates of identified Fungi isolates

PLATE 2: ***Aspergillus niger***

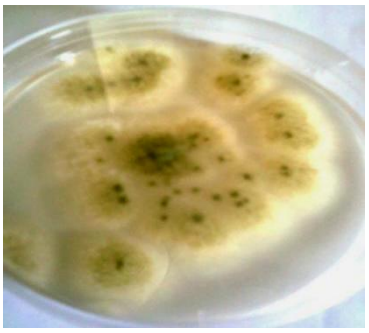


PLATE 3: ***Aspergillus flavus***

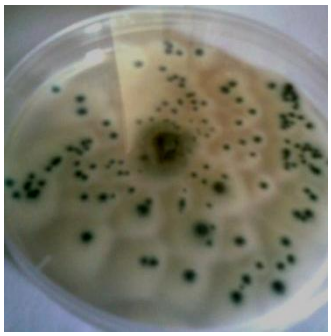


PLATE 4: ***Aspergillus fumigatus***

PLATE 6: ***Trichoderma species***

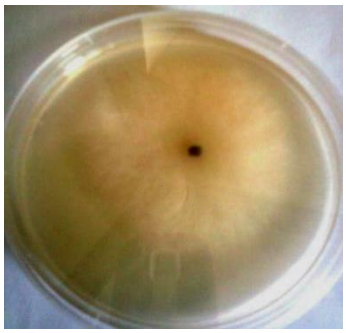
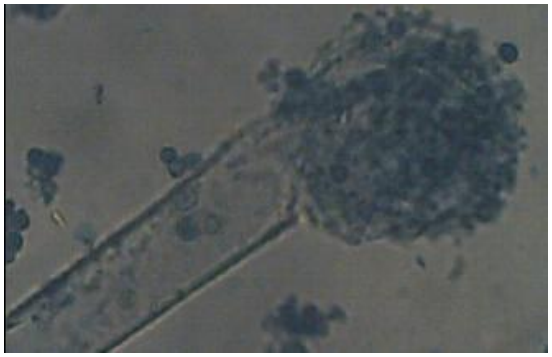


PLATE 6a: ***Rhizopus stolonifer*** **(young)**

PLATE 6b: ***R. stolonifer*** **(very old)**

PHOTOMICROGRAPHS OF IDENTIFIED FUNGI isolates



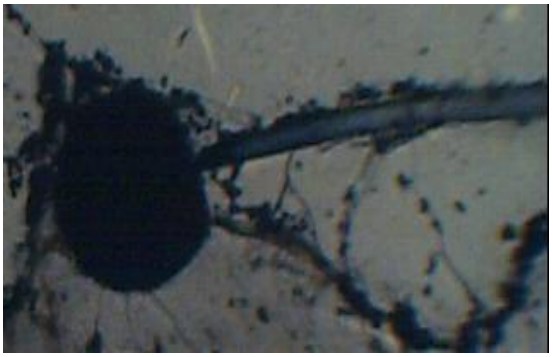


Plate 7: *A. flavus*

Plate 8: *A. niger*

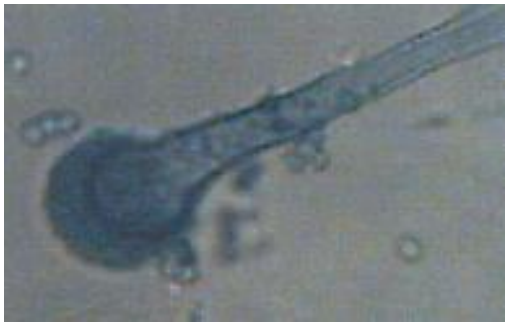


Plate9a: Typical conidiophores of *A. fumigatus*

Plate 9b: *A. fumigatus*

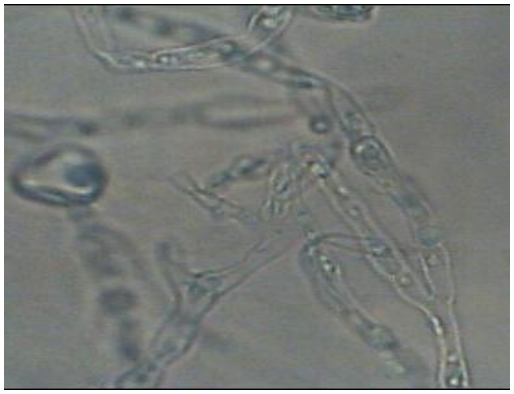


Plate 10: *R. stolonifer* (very old)

Plate 11: *T. spp*

Cellulolytic Assay

Two fungi isolates- *Trichoderma species* and *Aspergillus niger*, exhibited the best cellulolytic activity at both pH 4 and 5 of the CMC selective agar. A. niger had higher Cellulolytic activity at pH 4 with an ICMC value of 1.56 as compared to T. species with the value of 1.31 (Table 2; Plates 12&13). T. species on the contrary exhibited higher Cellulolytic activity at pH 5 with an ICMC value of 1.45 as compared to A. niger with the value, 1.25 (Table 3; Plates 17&18). No visible clearance zone was observed in the remaining fungi isolates (Plates 14-16, 19-21).

TABLE 2: Plate Screening Cellulolytic Assay on Selective CMC Agar at pH 4 (mean±SD)

s/n	Fungi strain	diameter of colony (cm)	clear zone diameter (cm)	I_{CMC} value
1	*T. species*	4.9 ± 0.05	6.47 ± 0.29	1.31
2	*A.* niger	4.53 ± 0.26	7.02 ± 0.15	1.56
3	*A. fumigatus*	8.30 ± 0.00	-	-
4	*Rhizopus stolonifer*	8.30 ± 0.00	--	-
5	*A. flavus*	8.30 ± 0.00	--	-

TABLE 3: Plate Screening Cellulolytic Assay on Selective CMC Agar at pH 5 (mean±SD)

s/n	fungi strain	diameter of colony (cm)	clear zone diameter (cm)	i_{cmc} value
1	*A* .niger	4.43 ± 0.93	6.08 ± 0.17	1.25
2	*A. flavus*	4.95 ± 1.13	ND	-
3	*T. species*	4.16 ± 0.29	6.02 ± 0.71	1.45

4	*A. fumigatus*	8.30 ± 0.00	ND	-
5	*Rhizopus stolonifer*	8.30 ± 0.00	ND	-

ND: not determined

Clearance Zones of Fungi Isolates at pH 4

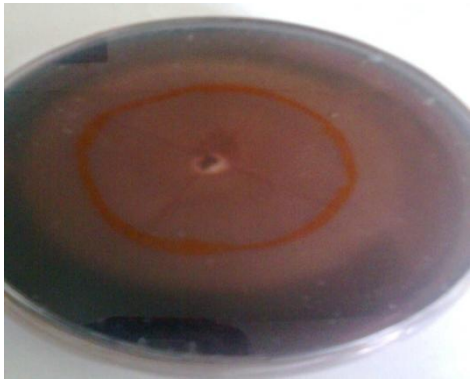


Plate 12: *T. species* at pH 4

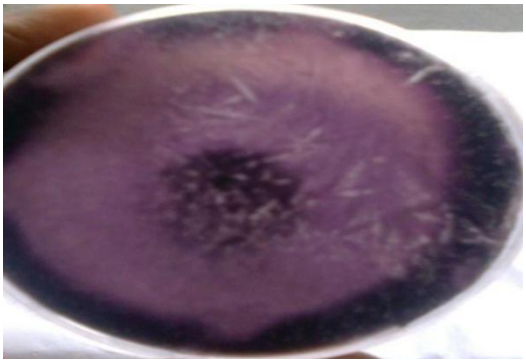


Plate 13: *A. niger* at pH 4

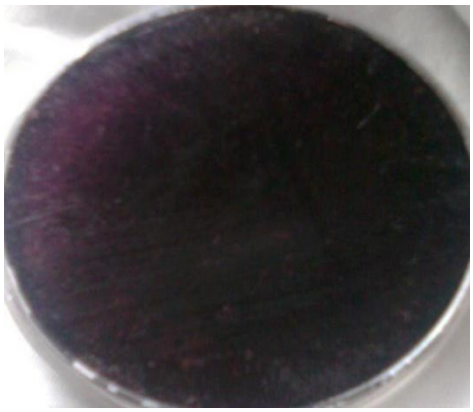


Plate 14: *A. fumigatus* at pH 4

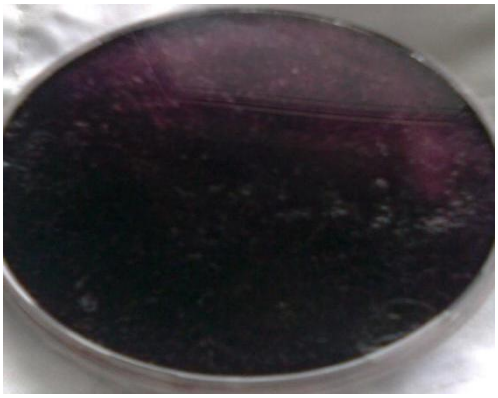


Plate 15: *A. flavus* at pH 4

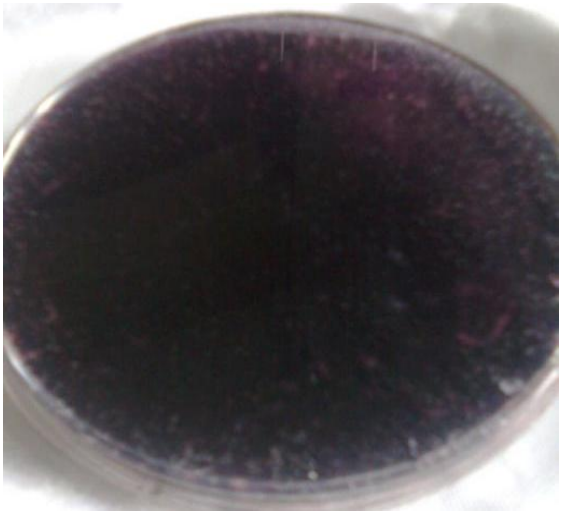


Plate 16: ***Rhizopus stolonifer* at pH 4**

Clearance Zones Fungi Isolates at pH 5

Plate 17: *A. niger* at pH 5

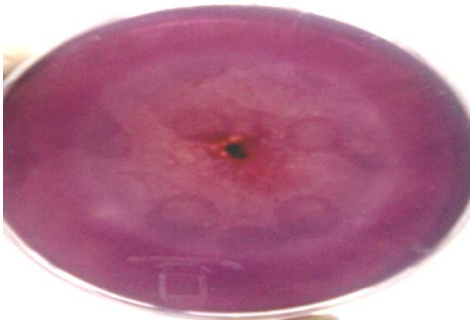


Plate 18: *T. species* at pH 5

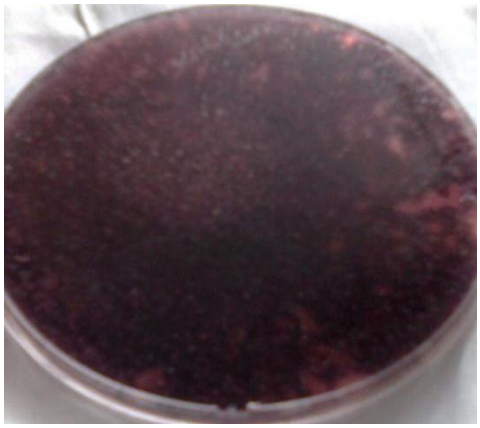


Plate 19: *Rhizopus stolonifer* at pH 5

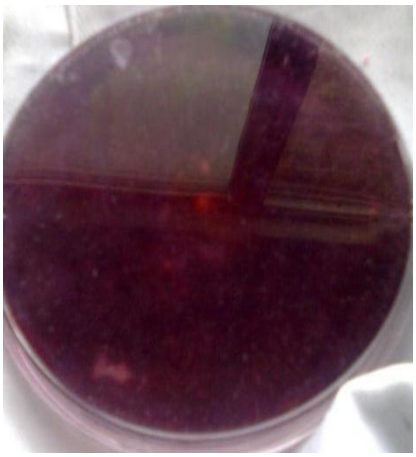


Plate 20: *A. flavus* at pH 5

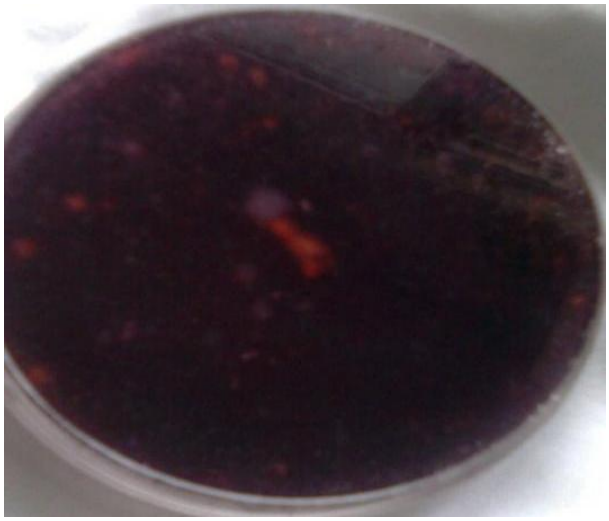


Plate 21: *A. fumigatus* at pH 5

INOCULA PRODUCTION

Pure white mycelia growths were observed in the inocula of *A. niger* and T. species after 20 days of incubation. *A. niger* had the characteristic black spore cover while T. species had a distinctive white spore cover as shown in Plates 15 & 16.

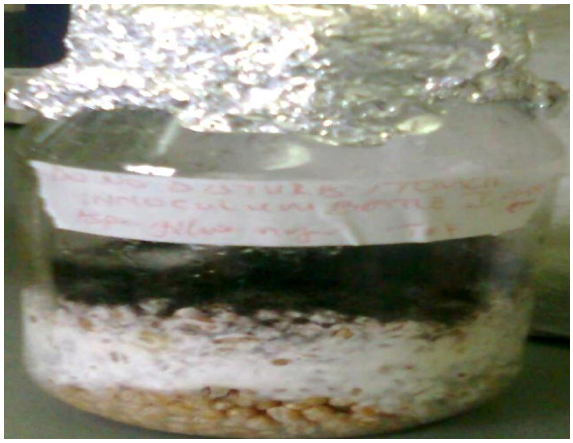


PLATE 15:Inocula of ***A. niger***

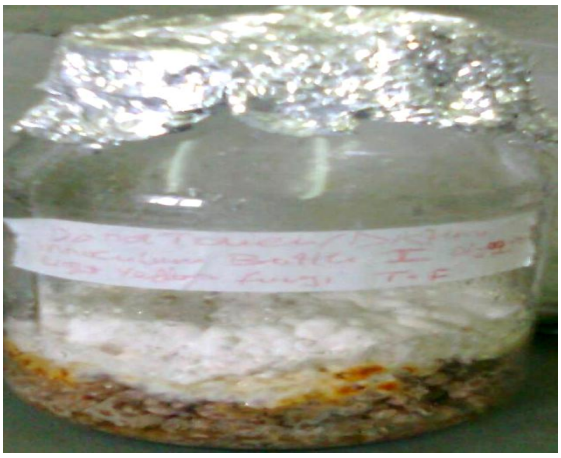


PLATE 16: Inocula **of *T. species***

Proximate Analysis

A considerable reduction was observed in the percentage (%) moisture, cellulose and lignin contents when comparing the fresh untreated waste (control) to the pretreated wastes with the lowest percentages (moisture- 82.44% cellulose-33.20% lignin- 13.46%) observed in the waste pretreated with both fungi (Table 4).

TABLE 4: Proximate Composition of Waste (Plantain Stalk) Before and after Pretreatment

Parameters	Fresh (Untreated Waste)	Pretreated[1]	Pretreated[2]	Pretreated[3]
% Moisture	90.34±0.19	92.36±3.68	89.72±0.47	82.44±1.02
Total solid (TS) (%)	9.66±0.20	10.26±0.64	10.29±0.47	18.20±0.62
Total Volatile solid (VS) (%)	84.55±2.09	77.69±3.75	80.07±0.37	87.72±0.82
Cellulose (%)	59.2±2.33	40.48±3.34	39.60±3.20	33.20±1.93
Lignin (%)	18.85 ±3.23	15.40±1.03	14.62±1.19	13.46±0.88
Hemicellulose (%)	15.00 ±4.00	10.3±4.11	16.67±1.25*	36.00±2.10*

1- waste pretreated with *A. niger* , 2- waste pretreated with *T. spp*, 3- waste pretreated with both fungi isolates (i.e. *A. niger* and *T. spp*)

*- percentage higher than Control, reason for high percentage not entirely known.

Discussion

Cellulose is the most abundant and renewable organic matter on earth (Omojasola and Jilani 2008; Shafique *et al.*, 2009). But its digestibility is hindered by its association with other complex compounds such lignin and hemicelluloses in form of lignocellulosic biomass (Howard *et al.*, 2003 ; Chukwuma *et al 2020*). Lignin, hollocellolose (hemicelluloses and cellulose), present in Lignocellulosic biomass, are the major energy sources available for decomposers (microorganisms) (Swift *et al.*, 1979 ; Su *et al* 2020).

The need for pretreatment in biogas production cannot be over emphasized. Paepatung *et al.* (2009) in their evaluation of the biogas potential of some selected feedstock, showed that the biodegradability and biogas yield of the selected feedstocks varied depending on the substrate composition, proportion of readily degradable and non-readily degradable organic compounds, such as lignin, cellulose and hemicellulose. They observed that the pineapple peel and cassava pulp had the highest biogas yield of 0.4 m^3 CH_4 per kg VS_{added} and 0.37 m^3 CH_4 per kg VS_{added} respectively over a period of 90 days retention time. The methane production from these feedstocks

slowed down after 10 days due to the slow degradation of complex organic compounds (cellulose, lignin and hemicellulose). Reters *et al.*, and Parawira *et al.*, as reported by Paepatung *et al.*, (2009) suggested that the reduction in methane yield could be as a result of inaccessibility of biodegradable compounds trapped within the cell wall. Paepatung *et al.* (2009) concluded that pretreatment processes will further enhance the methane yield of the feedstock.

Muthangya *et al.* (2009a) recorded a 24-30% increment in methane gas yield after pretreatment of Sisal Leaf Decortications Residues (SLDR) with isolates of *Trichoderma*, separately as compared to the untreated residue. Similar results were also observed when the residue was pretreated with the same isolates of fungi, in a two stage fungal pretreatment prior to anaerobic digestion (Muthangya *et al.*, 2009b).

Cellulases are a group of hydrolytic enzymes (Kader *et al.*, 1999 ; Ejaz *et al* 2021), which can be used for the pretreatment of lignocellulosic feedstocks for improved biogas production. Fungi are the main cellulase producing microorganisms, though some bacteria isolates have been reported to also yield cellulase activity (Immanuel *et al.,* 2007). Ahmad *et al.*, (2003) in their work using *Trichoderma harzianum* for the detection of cellulase enzyme production with different carbon sources, reported that CMC was the best carbon source for substantial enzyme production. CMC is a water soluble derivative of cellulose useful for the detection of cellulase production in micro-organisms (Mandels *et al.*, 1976 ; Nero *et al* 2022). The pH of the medium has a direct influence on the growth of the microbes carrying out the pre-fermentation process (Shafique *et al.*, 2009). Shafique *et al.* (2009) reported that maximum enzymatic activity was achieved at pH 4 on the CMC selective agar in their work indigenous *Trichoderma spp* of Pakistan. Similar results were also obtained by Suhr *et al.* (2002), with their work with *Penicillum spp.*

This contradicts the results obtained from this study, whereby, *A. niger* exhibited higher cellulolytic activity (1.556 I_{CMC} value) at pH 4 as compared to *T. sp* with 1.312 I_{CMC} value on the CMC selective agar. Hasper *et al.* (2002) got similar results in their work with *A. niger*. The optimum cellulolytic activity on CMC agar in their work with *A. niger* occurred at pH 4.5. Hankin and Anagnostakis (1977) observed that commercial cellulases derived from *T. viride* and A. *niger* produced distinctive hydrolyzing (clear) zones on CMC agar at pH 5 and 7 in their work with some species of bacteria and fungi. In general, according to Shafique *et al.* (2009), the physiological responses of same species of fungi may vary with ecological variations. This explains why variable enzymatic activities are observed between different and amongst the same fungi isolates. Although industrially produced enzymes from *A. niger* and *T. species* are available, the solid state fermentation method of pretreatment is still preferred over the use of industrial enzymes as reported in several works (Hankin and Anagnostakis 1977; Muthangya *et al.*, 2009b; Howard *et al.*, 2003; Xia and Len, 1999). This is as a result of the relatively high cost of enzyme production as reported by Xia and Len (1999) from the desired fungi strain.

Djarwanto and Tachibana (2010) noted that the waste degradation rate between fungi isolates varies. This supports the results obtained from this work with both *A. niger* and *T. spp* exhibiting variable degradation activities as observed from the analysis of the waste after pretreatment. The most dramatic reduction in lignin and cellulose content was observed when the waste was pretreated with both fungi isolates (in equal proportions) with a reduction of 28.59% and 43.77% respectively, followed by pretreatment with *T. spp* with 22.44% and 43.77% reductions and *A. niger* with 18.3% and 31.8% reductions respectively.

Thus, co- digestion or pretreatment of waste with two or more fungi, will be most favourable for the biological pretreatment of Lignocellulosic waste (agrowaste) prior biogas production (Muthangya *et al.* 2009a&b; Mshandete, 2008&2009). The variable cellulolytic activities observed in the fungi isolates at different pH is as a result of their differential physiological and metabolic activity.

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