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Maximizing Sustainability: Leveraging Indigenous Fungal Cellulases for Sugarcane Bagasse Bioethanol Production and Agricultural Waste Management in Kano

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ABSTRACT

This study aimed to investigate the potential of locally isolated cellulolytic fungi in the production of bioethanol from sugarcane bagasse. The research encompassed the identification of effective fungal isolates, the stimulation of cellulase production, the characterization and purification of these cellulases, and the assessment of their efficiency in the hydrolysis of pre-treated sugarcane bagasse to facilitate ethanol production via the Simultaneous Saccharification and Fermentation (SSF) process. In the pursuit of the most effective isolate, cellulolytic fungi were isolated from soil samples and subjected to a rigorous screening process. Subsequently, the chosen fungus was cultivated in an environment rich in cellulose to promote cellulase production.The purified cellulases exhibited a noteworthy enzymatic activity, measuring 0.108 FPU/ml. These meticulously purified cellulases were subsequently harnessed for the enzymatic depolymerization of pre-treated sugarcane bagasse. This process culminated in the generation of a hydrolysate instrumental in ethanol production via SSF, in conjunction with *Saccharomyces cerevisiae*.This study underscores the feasibility of utilizing locally isolated fungal cellulases to transform pre-treated sugarcane bagasse into a viable bioethanol feedstock. This study highlights the conversion of abundant agricultural waste, specifically sugarcane bagasse, into valuable bioethanol by leveraging cellulases from locally isolated fungi. This eco-friendly approach not only addresses waste management challenges but also provides a renewable and sustainable energy source. Particularly beneficial in regions with substantial sugarcane bagasse disposal, this research promotes cost-effective waste utilization and sustainable energy production.

Keywords:*Sugarcane bagasse, Cellulase, bioethanol, Pretreatment, Simultaneous Saccharification and Fermentation (SSF)*

1. Introduction

The global energy crisis remains a persistent challenge, intensified by the growing demand for energy driven by the increasing global population and the expanding scope of industrialization (Xu et al., 2022). The 1973 oil crisis, which resulted in an exponential spike in the price of oil, was one of the most significant episodes in the history of the energy crisis (Ramos et al., 2022). The main source of fuels addressing the escalating global energy demands are non-renewable fossil fuels like crude oil, natural gas, and coal (Kocak et al., 2021). Regrettably, these resources are finite and rapidly depleting, resulting in escalating costs.The rising amount of carbon dioxide released into the atmosphere, along with energy security concerns, has rekindled interest in renewable energy sources, which are considered a sustainable alternative (Martins et al., 2021). Wind, sun, geothermal, biomass, and hydropower are all examples of renewable energy sources. Investing in renewable energy can help to solve the world's energy challenge while also mitigating climate change and reducing our reliance on fossil fuels (Anser et al., 2022).

Biofuel production and consumption are cyclical and financially viable, resulting in zero net carbon dioxide emissions. To minimize the use of fossil fuels as an energy source, it is essential to rely on and exploit renewable energy sources (Ebabhi et al., 2019). Nigeria is rich in sugarcane bagasse, a fibrous residue that remains after juice extraction from sugarcane. With an estimated annual production of 600,000 to 720,000 tonnes, it has multiple uses in the country. One of its applications is to produce renewable energy by burning it to generate electricity through steam-powered turbines (Akintunde et al., 2022). Moreover, the fiber content of sugarcane bagasse makes it a valuable animal feed for ruminants like cattle and goats, providing energy and roughage. More so, it is a soil conditioner, enhancing soil structure, and water retention, and providing nutrients for plants (Awoyale et al., 2021). The pulp obtained from sugarcane bagasse can also be used to manufacture paper and paper-based products. Nigerian researchers and industries are exploring other potential uses of sugarcane bagasse to maximize its benefits (Awoyale & Lokhat, 2021).Cellulase is a critical enzyme in the production of ethanol from sugarcane bagasse since it facilitates the breakdown of cellulose into glucose, which can be fermented into ethanol by yeast (Huang et al., 2022). The process of ethanol production would be slower and less efficient without cellulase since yeast alone cannot break down cellulose. Therefore, cellulase plays a vital role in the conversion of cellulose to glucose and ultimately to ethanol (Abdulsalam et al., 2022). Blended lignocellulosic biomass, a mixture of various types of plant material containing lignocellulose, is more cost-effective and simplifies the management of feedstock supply variations compared to using a single substrate. Blending different types of lignocellulosic biomass optimizes feedstock composition, making it more suitable for the production of biofuels or other bioproducts (Bieleck & Zubkova, 2023). Moreover, blending increases feedstock supply flexibility, allowing for greater management of variations in the availability of different types of biomass.

Ethanol presents a promising biofuel option with the potential to supplant gasoline and alleviate existing and future fuel scarcity (Vaid et al., 2022). An area of active exploration lies in harnessing lignocellulose, particularly sugarcane bagasse, for ethanol production. The attractiveness of these substrates stems from their ready availability and cost-effectiveness,

positioning them as an enticing alternative to conventional ethanol production methods.Lignocellulosic biomass, a complex compound comprising cellulose, hemicellulose, and lignin, serves as the raw material (Anu et al., 2021). The conversion of lignocellulose into ethanol involves the breakdown of these intricate molecules into simpler sugars, which yeast can ferment to produce ethanol(Bieleck & Zubkova, 2023). Researchers continually seek ways to enhance the process's efficiency and reduce costs, striving to render it more competitive with established fuel sources(Abdulsalam et al., 2022).This research aims to look into using lignocellulose (sugarcane bagasse) to make ethanol. Despite the challenges, the utilization of lignocellulose for ethanol production has enormous potential in reducing dependence on fossil fuels and addressing the world's energy needs sustainably.

2. MaterialsandMethods

General procedures

All the glassware was washed, dried, and sterilized for 2 hours at 160°C in a hot air oven. Additionally, wire loops, inoculating needles, cork borers, beaker necks and mouths, and conical flasks were sterilized by flaming with a Bunsen burner. To maintain an aseptic work environment, 70% ethanol was used with absorbent cotton wool to wipe the working table before each experiment. Furthermore, all culture media utilized in the studies were prepared aseptically following the manufacturer's instructions. They were autoclaved for 15 minutes at 121°C before being dispensed into sterile plates or McCartney bottles to create slants.

Collection of samples (lignocellulosic biomass) of sugarcane bagasse

Sugarcane was obtained from the sugarcane market area in the Kano metropolis. It was chewed, and the resulting bagasse was sun-dried, milled, and passed through a 1 mm mesh. The biomass was stored at room temperature before the pretreatment process.

Isolation of cellulose-producing fungi from soil

The soil sample was collected from areas where trees had fallen and undergone degradation, and this was done during the cooler part of the day at around 10:00 a.m. The soil was then placed in a sterile polythene bag and kept before use. In the laboratory, a flask containing 50 ml of sterile distilled water and 5g of the soil sample was shaken vigorously to dislodge microorganisms into the medium. From this mixture, 1 mL was taken and serially diluted. To inhibit bacterial growth, 100µL aliquots of the serially diluted samples were introduced into culture plates containing sterile Potato Dextrose Agar (PDA) with streptomycin at a concentration of 0.1% (w/v). The samples were then spread uniformly on the surface of the medium using a sterile glass spreader. The culture plates were incubated in an inverted position at 25^{o}C for 48-72 hours to allow for the development of fungal colonies. Randomly picked fungal colonies that developed on the plates were purified by sub-culturing on PDA plates and then transferred onto PDA slants. These isolates were stored at 4^{o}C in the refrigerator as stock cultures for screening, production of fungal cellulose, and subsequent identification and characterization.

Identification of isolated fungi

Conventional identification: The identification of all fungal isolates was done conventionally using both microscopic and macroscopic views. The colonial characteristics used for identification included the appearance of the colony's surface and reverse side, the nature of the hyphae, pigmentation, opacity, margin or border, and elevation.

Molecular identification: The Molecular Biology Laboratory at the Bioscience Centre, International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria identified the best isolate for the production of fungal cellulase. The identification method utilized the sequencing of the genomic DNA's ITS1 and ITS2 regions, which were then compared to sequences of other fungi on the National Centre for Biotechnology Information (NCBI) to determine sequence similarity.

Plate screening (Primary screening)

The cellulolytic activity of each fungal isolate was confirmed through plate screening tests, following the methods of Kurnia & Astuti (2021) without grammatical errors. To the PDA medium, 0.5% (w/v) carboxymethyl cellulose (CMC) was added. Mycelia discs of 6 mm in diameter were taken from the edge of actively growing (3 days old) cultures of the isolates using a sterile cork borer. These discs were gently placed on the CMC agar plates and incubated at 25^{o}C for 48 hours. Following incubation, the plates were flooded with 1% (w/v) Congo red solution for 15 minutes. The stain was then discarded and the plate was later flooded with 1M NaCl for 15 minutes and then discarded. The cellulase-producing colonies appeared as pale orange to clear color against the dark red background.

Cellulaseproduction

Productionofextracellularenzymesinshakeflask: The plate-clearing zone assay was used to select fungal isolates for extracellular enzyme production. Submerged fungal cultures were used to induce extracellular cellulase enzymes. From five-day-old colonies of fungi cultivated at 25°C on PDA plates, agar blocks were inoculated into 250 mL conical flasks containing 100 mL of the medium used to assess cellulose digestion by specific fungi.The medium encompassed 1.4 g of $(NH_4)_2SO_4$, 2 g of KH_2PO_4, 0.3 g of Urea, 0.3 g of $CaCl_2$, and 0.3 g of $MgSO_4.7H_2O$, $5x10^{-3}$g $FeSO_4$, $1.6x10^{-3}$ g $MnSO_4$, and $1.4x10^{-3}$ g $ZnSO_4$were all present. As a carbon source, $2.0x10^{-3}$ g $CoCl_2$, 0.75 g Peptone, and 5 g L^{-1}amorphous cellulose were utilized per liter. The cultures were cultured in an orbital shaker incubator at 25°C for 5 days. Culture aliquots were centrifuged at 15000 rpm for 15 minutes at 4°C after 14 days of incubation to remove particles. The soluble protein and extracellular or free enzyme activity were evaluated after filtering the clear supernatant using a Whatman (GF/A) glass fiber filter.

Total cellulase activity (FPase assay)

The Wanget al. (2021) technique was employed to evaluate the total cellulase activity. A reaction mixture containing 0.5 ml phosphate buffer pH 5.8, a 1x6 mm filter paper strip, and 0.5 ml crude enzyme was incubated at 50°C for 1 hour in a test tube. The reaction was stopped by adding 3 ml

of dinitrosalicylic acid (DNS) reagent, and the mixture was boiled in a water bath for 5 minutes and then cooled in a cold water bath. The absorbance at 540 nm was measured using the Thermo Scientific Genesys-20 spectrophotometer. The amount of reduced sugar was determined using a curve generated by plotting the absorbance values against different glucose concentrations. One unit of filter paper cellulase activity (FPU) was defined as the amount of enzyme that produces 2.0 mg of reducing sugar from 50 mg of filter paper in 1 hour.

Pretreatmentoflignocellulosicbiomass

In a 250 mL stoppered flask, 5g of sugarcane bagasse sample was mixed with 100 mL of 1.0% (w/v) NaOH solution and autoclaved for 1 hour at 121°C. The mixture was then filtered through a muslin cloth to separate the solid residue. The residue was rinsed with distilled water until it reached a neutral pH, then air-dried and stored in a tightly sealed plastic bag in the refrigerator for future use (Basak et al., 2023).

BiomasshydrolysisandSSFforethanolproduction

The fungus-produced crude enzyme solution was utilized for enzymatic hydrolysis of the pretreated substrate. For the SSF experiments, the medium was prepared as described by Li *et al.* (2020), which included 5 g/L yeast extract, 1 g/L KH_2PO_4, and 0.3 g/L $MgSO_4.7H_2O$, along with 5% substrate loading and 10% (v/v) yeast inoculation in 100 mL Erlenmeyer flasks. Saccharomyces cerevisiae (Baker's yeast) was used as the yeast. Samples were taken at different time intervals (12h, 24h, 48h, 72h, and 96h) for ethanol assay, glucose concentration test, and growth determination, and were stored in the refrigerator.

Ethanoldetermination

The collected samples at various time intervals were centrifuged, and the resulting supernatant was used to determine the ethanol concentration. The ethanol concentration was measured using a modified version of the potassium dichromate method (Temporiti et al., 2022). Additionally, the DNS method was used to determine the glucose concentration in each sample. The spectrophotometer method was used to determine the dynamic profile of yeast growth.

3. Results

IdentificationofIsolates

Colonialmorphologyandmicroscopiccharacteristics

Twelve fungi were isolated from soil samples and identified using conventional techniques of microscopic examination and colonial morphology. The isolates were designated as F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, F_9, F_{10}, F_{11}, and F_{12}. The colonial morphology and microscopic characteristics of each isolate are presented below.

Isolate F_1:The hyphae were septate and hyaline. Conidiophores were simple, and phialides were grouped in brush-like clusters. The conidia were unicellular, round, hyaline, and had rough edges. The surface appeared powdery, orange to red, with a wrinkled radius furrow, and a heavy spore. This isolate was identified as *Penicillium* sp.

IsolateF_2:The growth is black and cottony, and it grows rapidly with radial furrows and a white/cream reverse. The hyphae are septate, and the conidiophores are unbranched. The conidiophore is enlarged at the tip, forming a swollen vesicle. It was identified as *Aspergillus* sp.

IsolateF_3:At first, it was white and cottony, but later it turned black, and it grew rapidly with a white reverse. The hyphae were septate, and the conidiophores were unbranched. The conidiophore enlarged at the tip, forming a swollen vesicle. It was identified as *Ulocladium* sp.

IsolateF_4: It grows rapidly and produces a green colony with a dark green reverse, a shallow center, and a radially furrowed raised margin with moderate spores, and white mycelium. It was identified as *Penicillium* sp.

IsolateF_5:The initial color was white, which later turned violet with discrete orange sporodochia. The sporodochia are transparent, flask-shaped projections, and the macroconidia are slightly curved, pointed at the tip, and non-septate. It was identified as *Fusarium* sp.

IsolateF_6:Initially, the isolate exhibited white growth, which later turned into blue-green or grey. In older cultures, the color changed to dark brown or brown-grey. The texture was velvety and flat, and the heads were columnar with a flask-shaped vesicle. The hyphae were present and septate, with a bluish-green color. Spores were also present, and the conidia heads were compact. The conidia were small, globose, and rough, and the phialides were uniseriate and evenly crowded together. White to yellow cleistothecia were also present. The colonies were granular and green in color, but they darkened with age. It was identified as *Aspergillus* sp.

IsolateF_7: It showed rapid growth, forming a greenish colony with irregular edges. The conidiophores were poorly defined and bright green, with a conspicuous conidial pigment. The fungus was identified as *Trichoderma* sp.

IsolateF_8:The colonies exhibit a white, fluffy, cotton-like growth with irregular margins. It was identified as *Sclerotinia* sp.

IsolateF_9:Initially, it appears white, but eventually, it develops yellow regions that turn black-brown to black due to conidia formation. The reverse side is wrinkled, translucent to yellow-white with wrinkled conidia heads. Radiating compacts of biseriate brown conidia tend to split as they age. The conidiophores are long, smooth, and aseptate, attached to separate hyphae through a foot cell. The conidia are dark and globose. It was identified as *Aspergillus* sp.

IsolateF_{10}:The colonies are black with rapid growth and black spores. They are radially furrowed with irregular edges and have a cream-colored reverse side. The conidia head is black and the spores are heavy. It was identified as *Aspergillus* sp.

IsolateF_{11}:The colonies had a surface that was wooly and rough, ranging in color from bright yellow-green to deep yellow-green. A variegated pattern was produced by the presence of dark red-brown to purple-red sclerotia. The conidiophores were long, non-septate, and hyaline, and had a distinctly roughened appearance. In young specimens, they were flask-shaped, while in

mature specimens the vesicles were globose or sub-globose. The fungus was identified as *Aspergillus* sp.

IsolateF_{12}:The colonies started as white and cottony and later developed speckles due to the growth of sporangia which turned black-brown. The growth was rapid and the colonies spread quickly. Upon microscopic examination, a distinct sporangium separated from the sporangiophore was observed along with columellate ovoid-shaped spores. The hyphae were non-septate and branched. The fungus was identified as *Rhizopus* sp.

The photomicrograph of the isolate is shown in Figure 1. Additionally, it reveals the microscopic characteristics of the sample such as its structure and cellular composition.

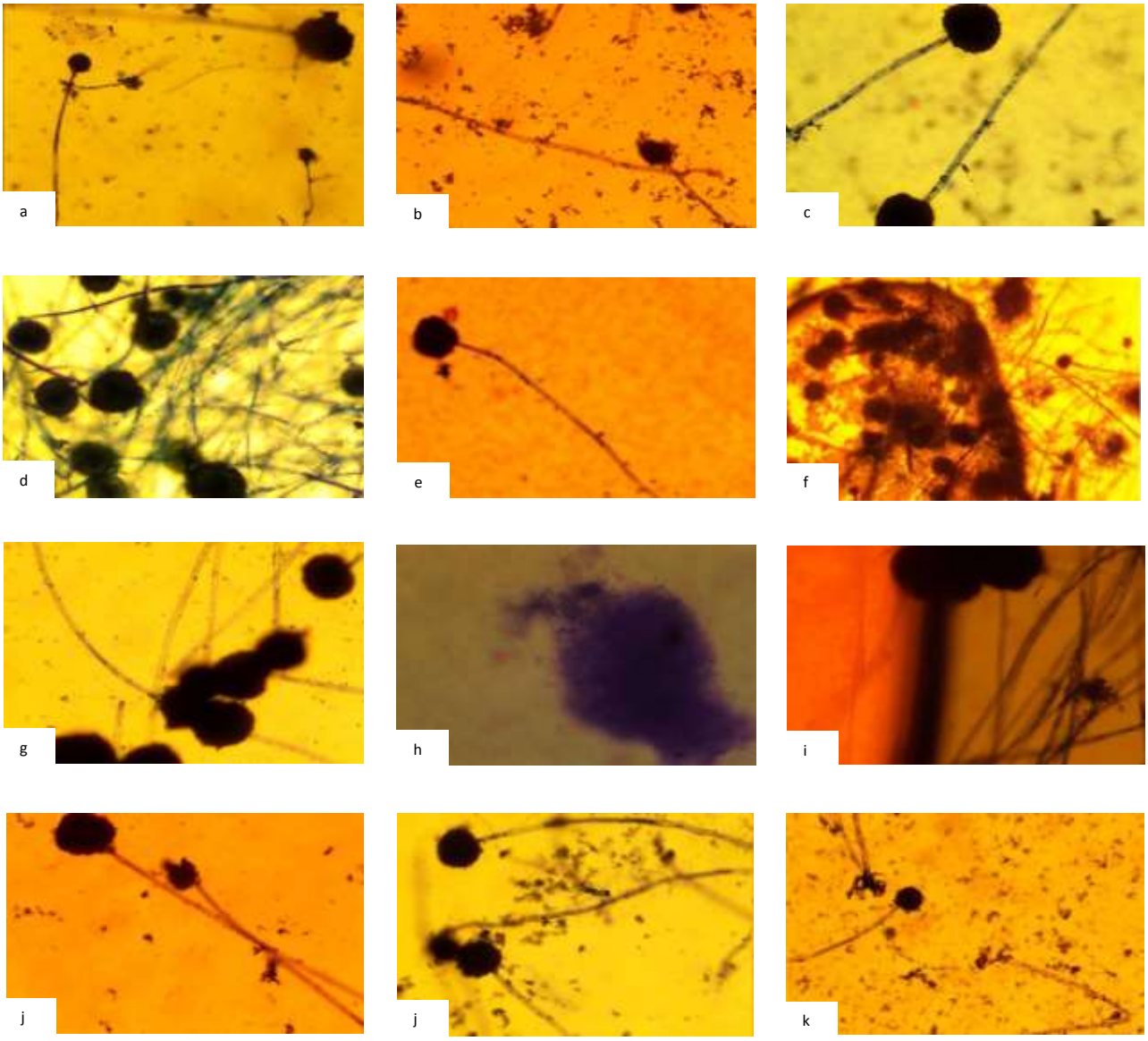

Figure 1:a. Microscopic view (100x) of F_1*Penicillium* sp., **b.** Microscopic view (100x) of F_2*Aspergillus* sp., **c**.Microscopic view(100x) of F_3*Ulocladium* sp., **d**. Microscopic view (100x) of F_4*Penicillium* sp., **e**. Microscopic

view (400x) of F_5*Fusarium* sp.,**f.** Microscopic view (400x) of F_6*Aspergillus* sp., **g**. Microscopic view (100x) of F_7*Trichoderma* sp.,**h**. Microscopic view (100x) of F_8*Sclerotinia* sp.,**i**. Microscopic view (100x) of F_9*Aspergillus* sp.,**j**. Microscopic view (400x) of F_{10}*Aspergillus* sp.,**k**. Microscopic view(400x) of F_{11}*Aspergillus* sp. and**1l.** Microscopic view (100x) of F_{12}*Rhizopus* sp.

Screeningoffungiisolates

Platescreening(Primaryscreening)

The fungal culture, isolated from soil, was screened for cellulolytic activity by observing the formation of clear zones on PDA plates supplemented with 0.5% carboxymethyl cellulose, as shown in Figure 1. The cellulolytic nature of the fungus (colony F_2) was confirmed by the maximal yellow zone produced around it, indicating its cellulolytic properties. The same culture was then used for cellulase production in submerged fermentation, and Table 1 presents the hydrolytic capacity measurements based on the clearance diameter around the colony. In Table 1, F_2 showed the highest hydrolytic capacity, while F_3 had the lowest hydrolytic capacity of 1.14.

Table1:Hydrolyticcapacityoffungalisolates

Isolates	Colonydiameter(mm)	Zoneofhydrolysis (mm)	Hydrolyticcapacity
F_1	4.00	5.00	1.25
F_2	1.00	3.00	3.00
F_3	7.00	8.00	1.14
F_4	4.80	5.50	1.15
F_5	4.80	5.50	1.15
F_6	-	-	-
F_7	2.90	3.80	1.31
F_8	-	-	-
F_9	3.50	4.76	1.34
F10	2.50	4.00	1.60
F11	4.00	5.80	1.45
F12	4.00	6.50	1.63

The isolate F_2, with the highest hydrolytic capacity, is the best candidate for ethanol production because it can efficiently break down complex carbohydrates into simple sugars. These simple sugars are then readily available for fermentation by yeast to produce ethanol. A higher hydrolytic capacity enables the extraction of more sugars from the same amount of starting material, leading to higher ethanol yield and possibly lower production costs. Thus, identifying isolates with high hydrolytic capacity is a crucial step in the development of effective and economical methods for ethanol production in Figure 2 (Ortega *et al*., 2022).

Table 2. Shows the molecular confirmation of isolate F2

Isolate	Organism	Number of Bases	Identity	Accession number
F2	*Aspergillus niger* ATCC 16888	576	99.0 %	NR_111348.1

Figure 2: Zone of Hydrolysis displayed by one of the fungal isolates

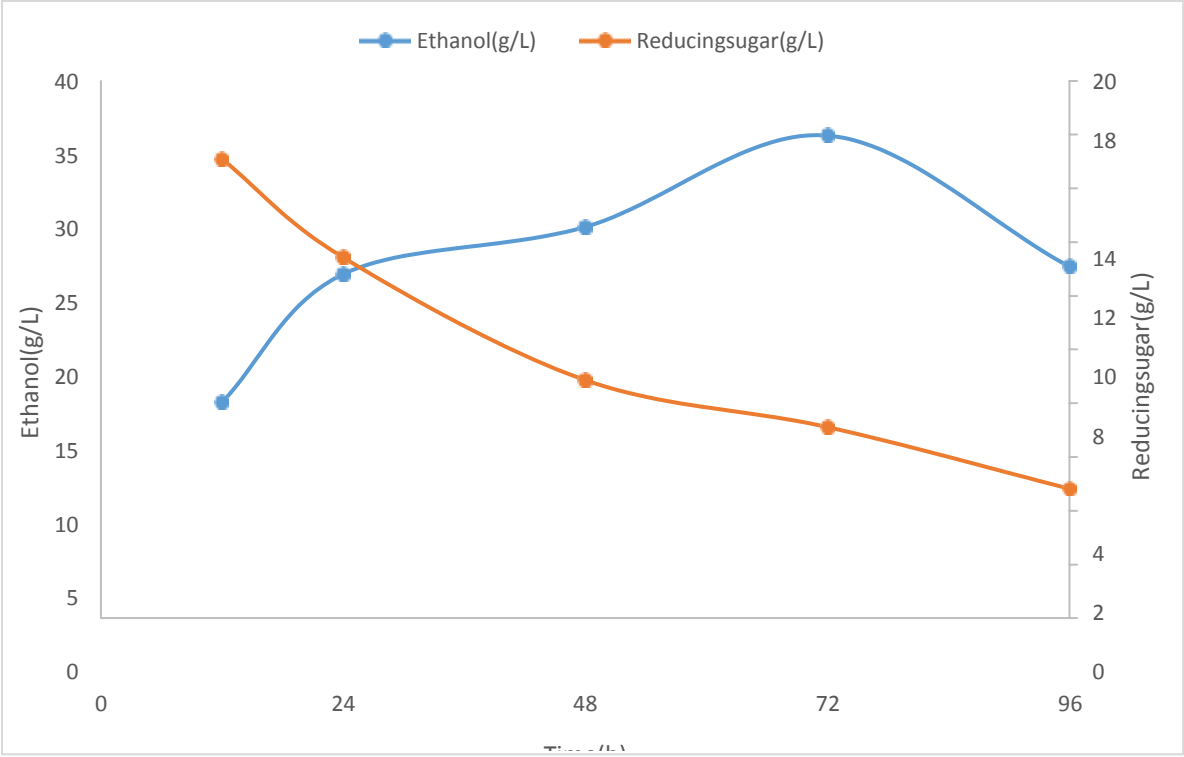

Figure3:Timecourseforsugarconsumptionandethanolproduction

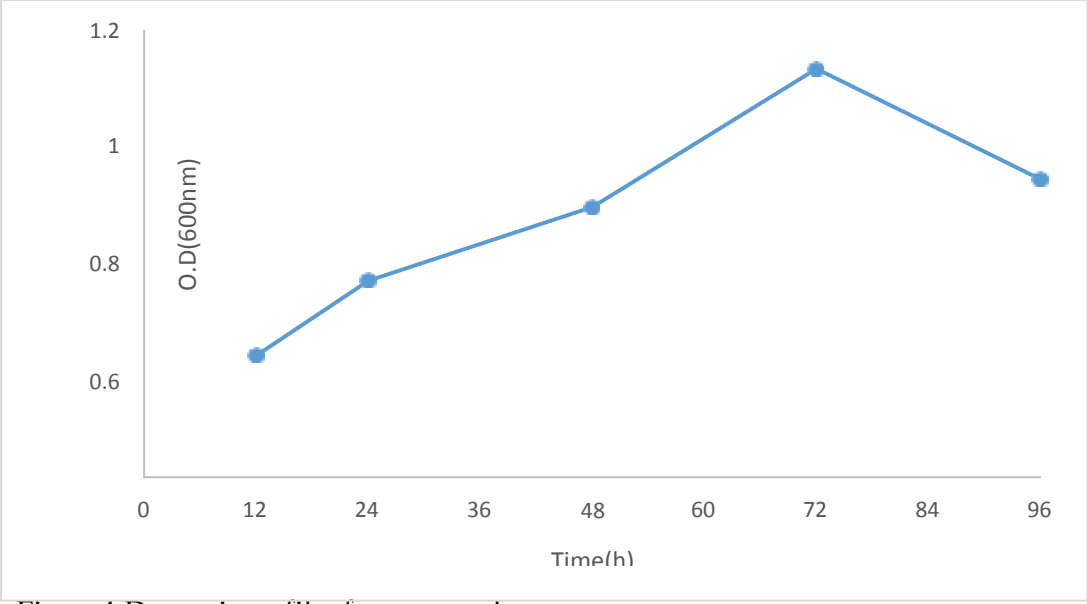

Figure4:Dynamicprofileofyeastgrowth

4. Discussion

Fungi are widely recognized for their ability to produce a variety of commercially valuable enzymes. Fungi are preferred over other microorganisms because they can be easily cultivated and produce a significant amount of extracellular enzymes that are important for industrial applications (Lopes et al., 2021). Several fungi species, including *Trichoderma* and *Aspergillus*, have been reported to synthesize enzymes at acceptable levels. These fungi thrive in the complex and diverse environment provided by soil microbial populations, as noted by Yunet al. (2022). Thus, understanding the role of fungi in soil ecosystems is crucial for the development of novel enzyme-based biotechnologies.

The soil sample yielded a total of twelve (12) fungal species, of which six were identified as members of the *Aspergillus* genus. This discovery aligns with the findings of Sadaf et al. (2015), who identified 61 *Aspergillus* species among 115 fungi isolated from soil, air, and diseased wheat plants.More so, various other fungal genera were revealed in this study, including *Penicillium* sp., *Fusarium* sp., *Ulocladium* sp., *Sclerotinia*, and *Rhizopus* sp. To definitively identify the fungal species, the ITS 1 region of genomic DNA was sequenced and compared to entries in the NCBI database, unveiling a striking 99% similarity to *Aspergillus niger* (Table 2). The prevalence of *Aspergillus* in the soil sample can likely be attributed to its remarkable adaptability to a wide array of environmental conditions, encompassing variations in soil pH, temperature, and nutrient availability, as highlighted by (Dusengemungu et al., 2022). Furthermore, *Aspergillus niger* is esteemed for its proficiency in generating an array of industrially significant enzymes like amylases, proteases, and cellulases, among others. These findings present a promising gateway to potential biotechnological applications involving this fungus, as explored by (Dey et al., 2012). From plate screening and FPase activity assays, ten fungal isolates exhibited potential for cellulase production, with a majority falling within the

Penicillium, Aspergillus, and *Trichoderma* genera. Among these isolates, *Aspergillus* sp. (F2) demonstrated the highest hydrolytic capability and was consequently selected as the most promising strain for cellulase production (Table 1). These findings support the research by Saliu and Sani (2012), who compared the crude cellulases of *Aspergillus niger* and *Penicillium decumbens*, revealing that *Aspergillus niger* displayed higher endoglucanase and total cellulase activity compared to *P. decumbens*. *Trichoderma* sp. is also widely recognized for its cellulase synthesis capabilities(Bansal et al., 2012). The production of fungal cellulase holds significant promise for various industrial applications, including biofuel production, bioremediation, and the pulp and paper industry(Dey et al., 2022). *Aspergillus* sp. (F2)'s remarkable cellulase production potential suggests its suitability for large-scale cellulase production(Dusengemunguet al., 2022).

According to Areeshi (2022), the utilization of purified substrates such as cellobiose, CMC, glucose, and cellulose for large-scale cellulose synthesis is not cost-effective. They compared the cellulase activity produced by *Aspergillus niger* using lignocellulosic biomass, including untreated sawdust (2.412 FPU/mL), jowar straw (1.52 FPU/mL), dry leaves (1.4 FPU/mL), and rice straw (0.96 FPU/mL), against purified substrates like glucose (0.154 FPU/mL), cellulase (1.632), cellobiose (1.021 FPU/mL), and CMC (1.040 FPU/mL). The type of media used and spore suspension employed for fermentation may have influenced the results. The use of spore suspension could also lead to the germination of more progeny, resulting in the production of more cellulase enzymes (Abdeljelil et al., 2023). This highlights the importance of selecting appropriate fermentation conditions to maximize cellulase production using lignocellulosic biomass as the substrate(Magagula et al., 2022).

The yeast *Saccharomyces cerevisiae* ferments the sugar produced from the enzymatic hydrolysis of the biomass mixture to produce ethanol (Bertacchi et al., 2022). When the biomass mixture was hydrolyzed with cellulases from *Aspergillus* sp., the highest amount of ethanol production was observed at 72 hours (Figure 3), resulting in a yield of 3.63% (36.30 g/L) ethanol which was almost twice the amount produced at 12 hours (Figure 4). In comparison, Hafid et al. (2016) obtained 0.45-0.5 g/L ethanol using a submerged fermentation involving *Saccharomyces cerevisiae*, *Candida parasitosis,* and *Lanchancea fermentation*. Other studies have shown that *Candida wickerhami*, *Pachysolen tannophylus*, *Saccharomyces cerevisiae*,*Kluyveromyces marxianus* var. *marxianus,* and *Kluyveromyces fragilis* can produce ethanol with conversion rates of 20%, 27%, 30%, 40%, and 45%, respectively (Umamaheswari et al., 2010). Additionally, when fermenting biomass hydrolysate at 60 g/L, the bacterium *Zymomonas mobilis* produced higher ethanol production (49%) compared to *Saccharomyces cerevisiae*, which only yielded 43% (Lawford & Rousseau, 2003).It is important to note that fermenting yeast requires sugar to produce ethanol and other metabolic products. Moreover, during the aerobic phase, the yeast begins to grow and consume some of the sugar before entering the anaerobic phase, which is characterized by ethanol generation(Tamis et al., 2021). The conversion efficiency of glucose to ethanol on a weight basis is 51%.

5. Conclusion

Fungi obtained from soil demonstrated the highest cellulose-producing activity, as evidenced by the formation of clear zones on carboxymethyl cellulose agar plates and their capacity to degrade filter paper. These fungal cellulases efficiently break down cellulose, releasing fermentable sugars. The study identified sugarcane bagasse as a promising energy feedstock, particularly for

bioethanol production. It also accentuates the perspective of soil-isolated fungi as a cellulase source for effective cellulose hydrolysis. Furthermore, exploiting sugarcane bagasse for bioethanol production presents a sustainable and cost-effective energy production approach. The study highlights the importance of exploring alternative and renewable energy sources, with microorganisms playing an essential role in this endeavor. Further research is required to optimize the fermentation process and investigate other potential biomass sources for bioethanol production.

Conflict of Interest

The authors declared no conflicts of interest.

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