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Classical Optimization of Cellulase and Xylanase Production by a Marine *Streptomyces* Species

Evelyn N. Fatokun [1,2,*], Uchechukwu U. Nwodo [1,2] and Anthony I. Okoh [1,2]

[1] Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa; unwodo@ufh.ac.za (U.U.N.); aokoh@ufh.ac.za (A.I.O.)
[2] SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice 5700, South Africa
* Correspondence: evelynfatokun@gmail.com; Tel.: +27-737896119; Fax: +27-866286824

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Abstract: Cellulase and xylanase are in high demand for application in several industrial processes, consequently necessitating the bioprospecting and manipulation of microbes for novel and greater enzyme productivity. This study reports on the optimal conditions for cellulase and xylanase production by a marine bacterial isolate from Nahoon beach sediment, via the classical process of one variable per time. Furthermore, the inducing effect of mono- and polysaccharides on enzyme production was investigated. The 16S rDNA gene sequence analysis clearly assigned the isolate to the genus *Streptomyces*, and was deposited at the GenBank under the accession number KU171373. Cellulase and xylanase production was optimal at the following conditions: pH 6 and 8, incubation temperature of 40 and 35 °C, and agitation speed of 100 and 150 rpm, respectively. Under optimum conditions, 0.26 U/mL and 18.54 U/mL activities were attained at 60 and 48 h with specific productivity of 205 and 7417 U/g for cellulase and xylanase, respectively. Xylanase production was induced by the entire mono- and polysaccharides tested, while cellulase production was induced by some. The results from this study signify the resourcefulness of the *Streptomyces* strain for production of cellulase and xylanase of industrial importance.

Keywords: cellulase; xylanase; optimization; saccharides; *Streptomyces*; activity; production

1. Introduction

Hydrolytic enzymes can be sourced from animals, plants, and microbes. However, microbial enzyme is favored for its higher scalability and malleability to industrial process conditions [1]. Hydrolytic enzymes include the cellulases and xylanases, which have gained increasing demand in several applications such as detergents production, processing in pulp and paper industry, as well as textiles, food, and feed industries [2]. In addition, lignocelluloses can serve as inexpensive resources for fermentable sugars and chemicals through the hydrolytic activity of these enzymes [3].

Cellulases and xylanases are inducible enzymes, usually synthesized by microbes when cultured on polysaccharide substrates, with lignocellulosic biomass as a foreseeable low-cost and readily available substrate for enzyme production [2,4]. The polysaccharide components of lignocellulose (LC) are cellulose and hemicellulose. Cellulose is a linearly arranged polysaccharide of glucose, with a repeating unit of cellobiose (disaccharide), connected through β-1,4-glycosidic bonds. Cellulose properties are influenced by the degree of polymerization and crystallinity. Intra- or intermolecular hydrogen bonds within the units form microfibrils which aggregate into high-order crystalline regions, or low-order amorphous regions, the former making cellulose a hydrolysis-resistant molecule [5]. Cellulase enzymes comprise endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21), which synergize in their activity for complete hydrolysis of cellulose. Hemicellulose polymers are classified according to the sugar residues of their backbone having xylan as

the major polymer. They comprise hexoses, including galactose and mannose; the pentoses, which are xylose and arabinose; and sugar acids (D-glucuronic acid) as monomeric units [6]. Xylanases are the enzyme complexes responsible for hydrolysis of xylan, and they include endo-β-1,4-xylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37), in addition to side-chain-hydrolyzing enzymes [6]. These plant cell wall hydrolases can be secreted within the intracellular region, having the activities within the cytoplasm, periplasm, and the cell membrane of the microbe; or secreted as extracellular enzyme which is released into the culture filtrate [7].

A major setback in the resourceful use of lignocellulosic biomass is a lack of efficient and cost-effective enzyme technology [8]. The high demand and cost of available commercial enzymes needed for efficient bioconversion of lignocelluloses makes the process economically nonviable [5]. Consequently, bioprospecting and manipulation of microbes for higher productivity and enhancement of existing commercial enzymes is of continuous interest [9,10]. Among other approaches, enzyme productivity can be increased by optimizing the production processes [8]. Optimization of cultural conditions is carried out so as to keep a balance in media composition, and favorable conditions for enhanced product formation [11]. Several investigations on varying cultivation conditions for optimal cellulase and xylanase production by different fungal and bacterial strains have been done; for instance, fungal genera including *Trichoderma*, *Penicillium*, and *Aspergillus* [12,13]; and the bacterial genera such as *Bacillus*, *Cellulomonas*, *Clostridium*, *Thermonospora* and *Arthrobacter* [14]. However, reports on marine bacterial isolates are an emerging area of interest [15]. The marine environments contain complex polysaccharides (CP) derived from sources including terrestrial and aquatic plants, algae, fungi, bacteria, and crustaceans; hence, it is a rich resource for microorganisms equipped with the enzymes battery for CP degradation [16,17]. Moreover, actinomycetes are microbial group not fully exploited for hydrolytic enzyme production despite their record of expansive metabolite production [18,19]. *Streptomyces* species are notable producers of several highly active secondary metabolites and extracellular enzymes [20,21]. This study reports on optimizing different culture conditions, and induction by some lignocellulose saccharides for cellulase and xylanase enzymes production by a *Streptomyces albidoflavus* strain isolated from marine beach sediments.

2. Materials and Methods

2.1. Source of Bacteria

The bacterial strain was isolated from sediments of Nahoon Beach located in East London, Eastern Cape South Africa, by stamping method in accordance with Mincer et al. [22]. The isolate was screened and selected based on its lignocellulolytic potential following the method of Maki et al. [1].

2.2. Identification of the Bacterial Isolate by 16S rDNA

Phylogeny of the bacterial strain was determined by sequence investigation of 16S rDNA gene. DNA extraction and polymerase chain reaction (PCR) amplification were carried out according to Cosa et al. [23]. Briefly, about 2–3 bacterial colonies were placed in 70 µL of sterile ddH_2O and agitated to form a suspension. The suspension was heated in a heating block at 100 °C for 10 min, cooled for 5 min and centrifuged at slow speed of 4500× *g* for 5 min. The supernatant so obtained was transferred into a sterile Eppendorf tube and stored at 4 °C. This served as the template in the PCR protocol. Amplification by PCR was carried out using the forward and reverse universal gene primers (F1: 59–AGAGTTTGATCITGGCTCAG–39 and R5: 59–ACGGITACCTTGTTACGACTT–39, respectively; I = inosine) and 2 µL of template DNA [23]. Almost full-length 16S rDNA sequences were amplified using this primer. The PCR amplicon was confirmed by visualizing on 1% agarose gel electrophoresis, and sequenced thereafter at University of Kwa-Zulu Natal, Durban (South Africa). Sequence analysis of PCR product was carried out with the Basic Local Alignment Search Tool (BLAST) algorithm system of National Centre for Biotechnology (http://www.ncbi.nlm.nih.gov/Blast.cgi). A 98% similarity sequence match with GenBank database was selected using the FASTA format and

subjected to phylogenetic analyses in MEGA6 software using the neighbor-joining method based on p-distance model to determine the evolutionary distance [24,25].

2.3. Media Composition and Fermentation Conditions

Enzyme production was carried out under submerged fermentation using minimal medium with the following composition (g/L): 0.1% KCl, 0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% yeast extract, supplemented with 1% carboxymethyl cellulose (CMC) (Merck, Modderfontein, South Africa) or beechwood xylan (ROTH, Cape Town, South Africa) as carbon source for cellulase and xylanase production, respectively. The initial pH of the medium was adjusted to 7.0 and sterilized by autoclaving at 121 °C for 15 min [1]. An inoculum was prepared by placing two loopful of the bacterial cells in a 25 mL of minimal medium, and incubated at 30 °C for 48 h. Subsequently, 1 mL of preculture adjusted to 0.1 OD_{600} was used to inoculate 100 mL of production medium and incubated in an orbital shaker, at 140 rpm, 30 °C for 72 h, along with control culture of an uninoculated 100 mL fermentation broth. Thereafter, the fermentation broth was then centrifuged at 15,000× *g* for 15 min at 4 °C and the supernatant obtained served as crude extracellular enzyme solution [26].

2.4. Determination of Optimal Parameters for Enzyme Production

Various cultivation conditions were adjusted for optimal enzyme production, using the classical approach of one variable per time, whereby a single culture condition is adjusted while keeping others constant [27]. Optimized conditions were then used sequentially in the subsequent fermentation.

2.4.1. Effect of Initial pH on Cellulase and Xylanase Production

In optimizing for pH, the fermentation medium was adjusted to initial pH range of 3–11 with 1N NaOH or 1N HCl as the case may be, before autoclaving. The sterilized fermentation medium was inoculated with 1 mL of 48 h cell suspension and incubated for 72 h.

2.4.2. Effect of Incubation Temperature on Cellulase and Xylanase Production

For optimum temperature determination, the fermentation medium was inoculated and incubated at varying temperature range from 25 to 50 °C. Thereafter, the medium was centrifuged and the cell-free culture supernatant served as crude enzyme.

2.4.3. Effect of Agitation Speed on Cellulase and Xylanase Production

In determining the optimal agitation speed for enzyme production, the inoculated and control fermentation medium were incubated under static condition, and at varying agitation speed of 50–200 rpm with magnitude interval of 50 rpm.

2.5. Time Course and Growth Kinetic for Production of Cellulase and Xylanase

Growth kinetics of the *Streptomyces* strain and enzyme production over time was also investigated. Briefly, 1 mL of 48 h preculture cell suspension at 0.1 OD_{600} was inoculated into 100 mL of fermentation broth, and incubated under optimized conditions for 96 h. The fermentation broth was intermittently withdrawn at 12 h intervals to determine enzyme activity and monitor bacterial growth. Quantitation of growth was by microbial biomass determination in which the culture broth was centrifuged at 15,000× *g* for 15 min at 4 °C, and the cell pellets were washed with sterile distilled water and oven-dried at 80 °C to a constant weight. Microbial biomass was reported as the dry cell weight (g/L) of culture broth; and enzyme productivity per microbial biomass as specific productivity (U/g) [28].

2.6. Inducing Effect of Some Mono- and Polysaccharides on Enzyme Production

Selected carbon sources at a concentration of 1% (*w*/*v*) were used to supplement the basal medium in order to determine their effectiveness in inducing holocellulase (xylanase and

cellulase) production by the *Streptomyces* strain [29]. The monosaccharides solutions were autoclaved separately and were aseptically mixed with sterile basal salts medium before inoculating with the bacterial suspension. Selection of carbon source was based on monomeric and oligomeric saccharides of lignocellulose. In addition to CMC and beechwood xylan, the following carbon sources were tested: microcrystalline cellulose (Avicel®, Sigma-Aldrich, Darmstadt, Germany), cellobiose (ROTH, Cape Town, South Africa), mannose, arabinose (Sigma-Aldrich, Darmstadt, Germany), galactose, D-glucose, D-xylose (Merck, Modderfontein, South Africa), and two types of lignocellulosic wastes biomass: wheat straw and saw dust. The saw dust was locally sourced from a wood factory (Melani village, Alice, Eastern Cape, South Africa). The wheat straw was obtained from farm field (agricultural farm of the University of Fort Hare, Alice, Eastern Cape, South Africa), thoroughly washed with distilled water, oven-dried at 60 °C for 48 h, pulverized with a laboratory mill (Lasec, Cape Town, South Africa) to 2 mm size particles and stored in air-tight plastic until further use [30]. Fermentation was carried out for 72–96 h; thereafter the broth was centrifuged and assayed for extracellular cellulase and xylanase activity.

2.7. Enzyme Assay

The enzyme activity was determined according to Saratale et al. [31], with slight modification. Quantitation of reducing sugar released from CMC and xylan was carried out by the 3,5-dinitrosalicylic acid (DNS) method [32], using D-glucose and D-xylose as standard for cellulase and xylanase respectively.

Cellulase assay: Cellulase activity was determined by a reaction volume comprising 1 mL of crude enzyme and 1 mL of 1% (*w*/*v*) CMC dissolved in 50 mM phosphate buffer (pH 7.0), which was incubated in a water bath (Lasec, Cape Town, South Africa) at 50 °C for 30 min.

Xylanase assay: Xylanase activity was carried out by mixing 1 mL suitably diluted enzyme with 1 mL of 1% (*w*/*v*) beechwood xylan, dissolved in 50 mM phosphate buffer (pH 7.0). The reaction mixture was incubated for 10 min at 50 °C.

Reaction tubes were incubated with controls and sugar standards. All reactions were terminated by addition of 2 mL 3,5-dinitrosalicylic acid reagent, placed in boiling water bath for 10 min and cooled in ice water. The absorbance was read at 540 nm. One unit of enzyme was defined as the amount of enzyme per milliliter needed to liberate 1 µmol of reducing sugar from a substrate per minute under the stated assay conditions.

2.8. Statistical Analysis

All experiments were run in triplicates and data obtained were analyzed for the average and standard deviation in Microsoft Excel spreadsheet, and subjected to one-way analyses of variance (ANOVA) using the Statistical Package for Social Sciences (IBM SPSS Statistics 23; IBM Corp, Armonk, NY, USA) to determine the Fisher's least significant difference between variable mean. A $p < 0.05$ between the variables was considered significant.

3. Results

3.1. Identification of Bacterial Strain

This study reports on bacterial isolate designated as FS1k, which was selected on the basis of its lignocellulolytic potential, and cellulase and xylanase production. The BLAST analysis of the 16S rDNA sequence clearly assigned the isolate to the *Streptomyces* genus, with 98% similarity with *Streptomyces albidoflavus* strain AIH12 (Figure 1), and was assigned the nomenclature of *Streptomyces albidoflavus* strain SAMRC-UFH5 and deposited at the GenBank under the accession number KU171373.

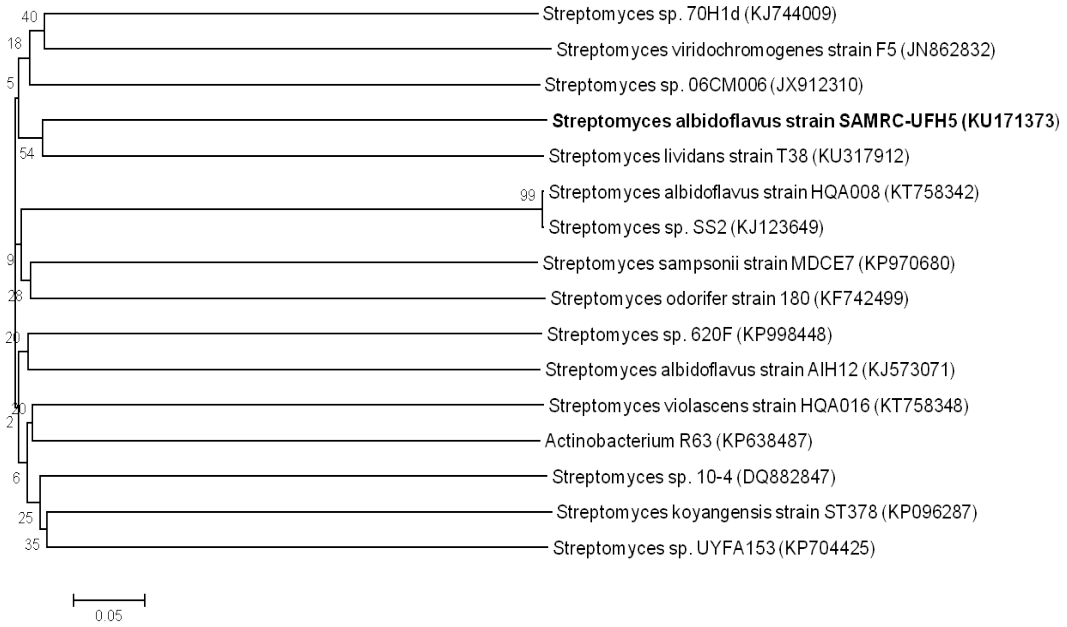
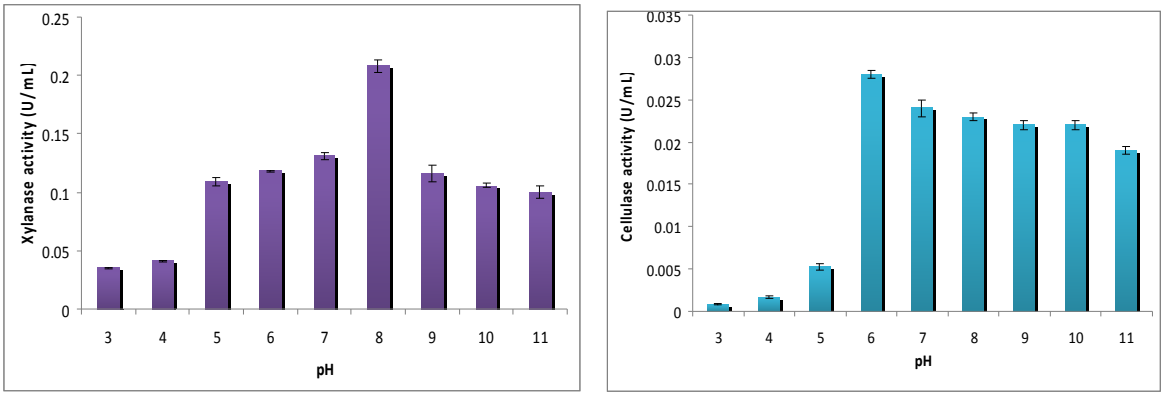


Figure 1. Unrooted phylogenetic tree construct showing the relationship of *S. albidoflavus* strain SAMRS-UFH5 (bold face) with selected actinomycetes in GenBank database. The Bootstrap values of 1000 replicates are shown next to the branches. The scale bar indicates the number of substitutions per site.

3.2. Effect of Initial pH of Fermentation Medium on Cellulase and Xylanase Production

The result of investigation carried out in this study on effect of initial pH on cellulase and xylanase production by *Streptomyces albidoflavus* strain SAMRC-UFH5 is depicted in Figure 2a,b. Xylanase production by the *Streptomyces* strain was over a wide pH range but optimal at pH 8, with an activity of 0.21 ± 0.01 U/mL and the least being 0.035 ± 0.001 U/mL at pH 3. While there was statistical difference between the pH range ($p < 0.05$), none was observed between pH 5, 6, 9 and 10, (Figure 2a). On the other hand, cellulase was produced optimally at pH 6 with an activity of 0.028 ± 0.001 U/mL and only a slight decline, with no significant difference between production, at pH 8–10; while the least production was observed at pH 3 (Figure 2b).



(**a**) (**b**)

Figure 2. Effect of initial pH on xylanase (**a**) and cellulase (**b**) production. Error bars represent standard deviation (SD) from the mean of triplicate tests.

3.3. Effect of Incubation Temperature on Cellulase and Xylanase Production

The effect of temperature on cellulase and xylanase production by *S. albidoflavus* strain SAMRC-UFH5 was examined and is shown in Figure 3. Although optimal xylanase production was recorded at temperature of 35 °C with activity of 3.47 ± 0.1 U/mL, production was also recorded at all the incubation temperatures tested. Moreover, there was no statistical significance between xylanase production at temperatures of 25, 30, and 40 °C, and between 45 and 50 °C. On the other hand, cellulase was optimally produced at 40 °C, followed by 35 °C with respective activity of 0.011 ± 0.001 and 0.009 ± 0 U/mL. Other temperatures at which cellulase production was recorded in order of descent are: 30, 25, 45, and 50 °C with activity of 0.002 ± 0, 0.001 ± 0, and 0.0003 ± 0 U/mL respectively; however, there was no significant difference between cellulase production at 25 and 30 °C (Figure 3b). Moreover, a sharp decline of over 50% in production of both cellulase and xylanase was observed with further increase in the temperature to 50 °C.

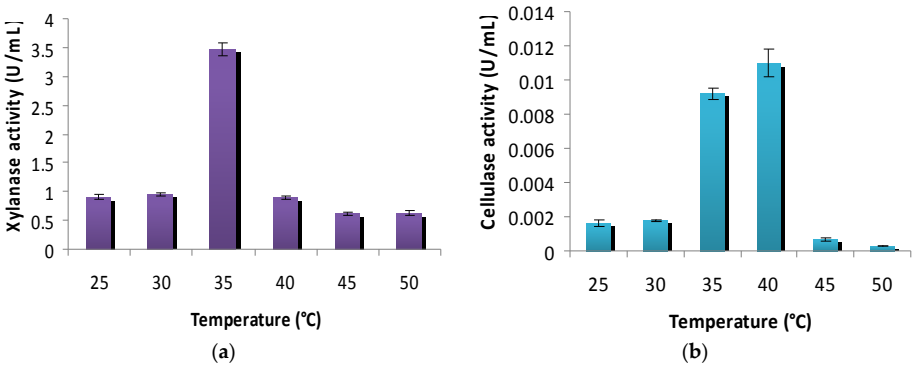


Figure 3. Temperature effect on xylanase (**a**) and cellulase (**b**) production. Error bars represent standard deviation (SD) from the mean of triplicate tests.

3.4. Effect of Agitation on Cellulase and Xylanase Production

The influence of static and varying agitation speed on cellulase and xylanase production was examined and the result is presented in Figure 4. Highest xylanase production by the *Streptomyces* strain was attained at agitation speed of 150, followed by speed of 100 (rpm), with respective activities of 4.02 ± 0.02 and 2.44 ± 0.02 (U/mL). Although the lowest production was recorded under static conditions, there was no significant difference between xylanase production at static (0) and 200 (rpm). A similar trend was observed for cellulase production, although the highest activity of 0.013 ± 0 U/mL was attained at 100, followed by 0.007 ± 0.001 U/mL at 150 (rpm); the lowest production of cellulase was also observed under static (0) condition (0.004 U/mL).

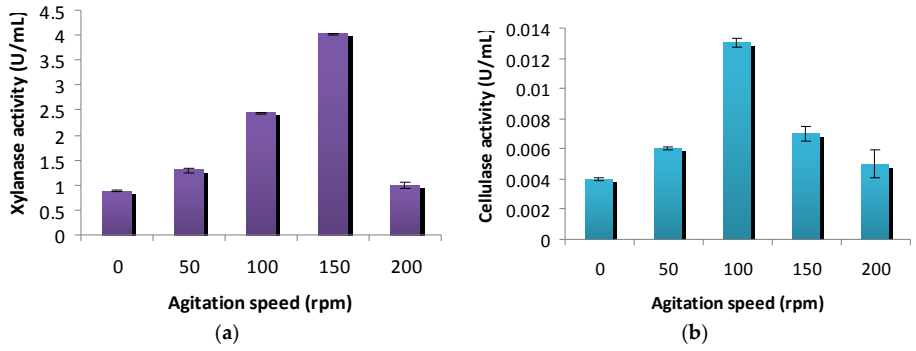
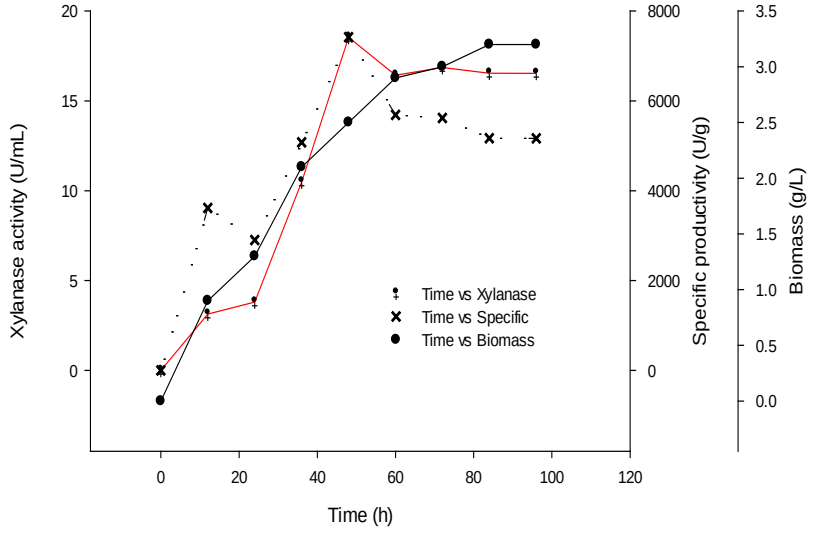


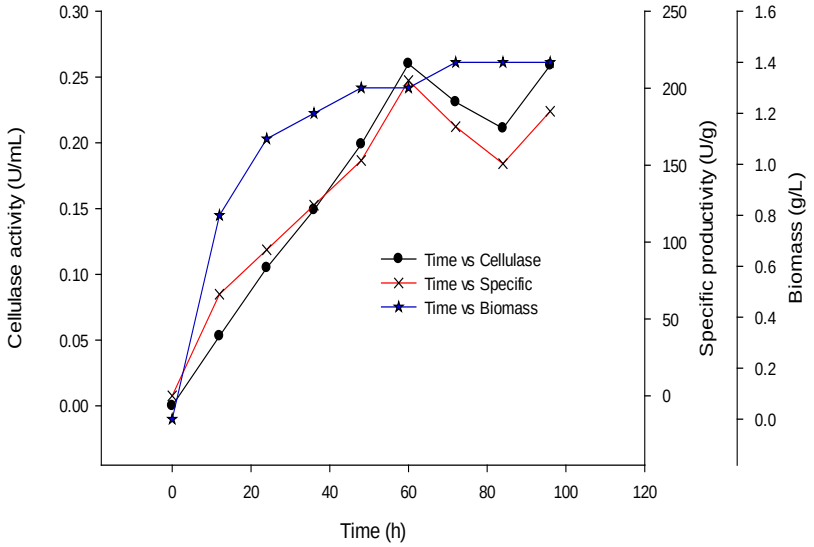
Figure 4. Production of xylanase (**a**) and cellulase (**b**) under static and different agitation speed. Zero (0) speed represents static condition. Error bars represent standard deviation (SD) from the mean of triplicate tests.

3.5. Time Course and Kinetics for Production of Cellulase and Xylanase

The *Streptomyces* strain was cultured under optimized conditions and monitored for growth, in addition to cellulase and xylanase production, for a period of 96 h. Production was initiated at 12 h of incubation, corresponding to early logarithmic growth phase (Figure 5a,b). The result also indicated that the *Streptomyces* strain attained optimum xylanase production at 48 h with an activity of 18.54 ± 0.58 U/mL and specific productivity of 7417 ± 232 U/g (Figure 5a), which also corresponded with the late logarithmic growth of the strain. Growth and xylanase production were in tandem up until 60 h, after which there was a decline in xylanase production, with specific productivity of 5688 ± 124 U/g (Figure 5a). On the other hand, optimal cellulase production by the *Streptomyces* strain was attained at 60 h with activity of 0.27 ± 0.01 U/mL, and a specific productivity of 205 ± 14 U/g also at late logarithmic growth phase. A decline in cellulase production was observed at 72 and 84 h with specific productivity of 175 ± 9 and 151 ± 12 U/g, respectively; however, a slight increase was recorded at 96 h (185 ± 2.8 U/g) corresponding to stationary growth phase (Figure 5b).



(**a**)



(**b**)

Figure 5. Growth and kinetics of xylanase production (**a**) dots: time vs. xylanase activity; cross: time vs. specific productivity; closed circle: time vs. biomass; and cellulase production (**b**) closed circle: time vs. cellulase activity; cross: time vs. specific productivity; asterisk: time vs. biomass.

3.6. Inducing Effect of Carbon Source Inducers on Holocellulase Production

The effect of different LC wastes and carbon components on inducing cellulase and xylanase production by the *Streptomyces* strain was examined and is summarized in Table 1. Cellulase production was induced by three of the carbon sources; CMC, arabinose, and mannose with activities of 0.070 ± 0.01, 0.061 ± 0.01, and 1.33 ± 0.01 U/mL respectively. In addition, wheat straw slightly induced cellulase production, with an activity of 0.01 ± 0. On the other hand, xylanase production was induced by the entire carbon sources, and wheat straw had the highest inducing effect, followed by beechwood xylan, galactose, and xylose with activities of 14.86 ± 0.56, 14.15 ± 0.07, 10.35 ± 0.65, and 8.02 ± 0.65 U/mL, respectively. Whereas wheat straw induced the highest xylanase production by the *Streptomyces* strain with slight cellulase activity, there was no significant difference between xylanase activity detected in the wheat straw and xylan cultures, although no cellulase activity was detected in culture of the latter. Moreover, CMC, arabinose, mannose, and wheat straw induced production of both cellulase and xylanase as indicated by cellulase and xylanase activities in the culture supernatant with statistical significance ($p < 0.05$) between xylanase and cellulase production (Table 1). In addition, low xylanase activity of 0.17 ± 0.01 U/mL was detected in the control basal medium which had no mono- or polysaccharide carbon source; however, no cellulase activity was detected.

Table 1. Carbon source inducers of cellulase and xylanase production.

Carbon Source	Cellulase Activity (U/mL)	Xylanase Activity (U/mL)
Monosaccharides		
Arabinose	0.061 [b] ± 0.01	0.30 [a] ± 0.01
Galactose	ND	10.35 [a] ± 0.65
Glucose	ND	2.55 [a] ± 0.36
Mannose	1.33 [b] ± 0.014	5.92 [a] ± 0.57
Xylose	ND	8.02 [a] ± 0.65
Disaccharide		
Cellobiose	ND	4.39 [a] ± 0.09
Polysaccharide		
Avicel®	ND	1.66 [a] ± 0.13
CMC	0.070 [b] ± 0.01	2.58 [a] ± 0.15
Xylan	ND	14.15 [a] ± 0.07
Lignocellulose		
Wheat straw	0.01 [b] ± 0.002	14.85 [a] ± 0.56
Saw dust	ND	2.29 [a] ± 0.35
Control *	ND	0.17 ± 0.01

[a,b] Row mean with significant difference; * Basal medium without carbon source; ND: not detected.

4. Discussion

In view of the increasing demand for cellulases and xylanases and the consequent need for higher productivity, it becomes very vital to source for novel enzymes and enzyme-producing microbial strains [9]. In that regard, for a thriving industrial production of cellulases and xylanases enzymes, utmost consideration is given to the selection of microorganism and the process strategies [9]. The genus *Streptomyces* has been implicated over the years as a contraption of several metabolites and biomolecules, including hydrolytic enzymes [21,33]. However there has been scant report on hemicellulolytic potential of *Streptomyces albidoflavus* [34,35]. Microbial hydrolytic enzyme production is a multivariable controlled process involving the composition and microbial cell culture conditions. Therefore, quantifying the influence of these variables is vital for an enhanced enzyme titer and productivity [10].

4.1. Effect of Initial pH of Fermentation Medium on Cellulase and Xylanase Production

Many enzyme systems and transport of several enzyme species across the cell membrane are influenced by the initial pH of cultivation medium [36]. In addition, synthesis and expression of certain genes, as well as microbial metabolic activities, are thought to be influenced by the internal pH, in response to that of the external environment [37]. Consequently, bacterial synthesis and activity of proteins associated with various processes are adjusted in response to internal and external pH levels [37]. The findings of this study were in accordance with an earlier report by Tuncer et al. [38], but contrary to the optimal pH 4 for xylanase production by *Streptomyces albidoflavus* PSM-3n reported by Sharma et al. [26]. Optimal initial pH for cellulase and xylanase production varies between bacterial groups, due to differences in interactions of media compositions and microbial specificities [39]. For instance, optimum pH 6.5 and 8 for cellulase production by *Streptomyces* strain C188 and *Streptomyces* sp., respectively, have been reported [28,40], while Acharya and Chaudhary [41] reported pH 6.5 as optimal for CMCase production by both *B. licheniformis* MVS1 and *Bacillus* sp. MVS3. In addition, other authors reported optimal initial pH 7 for xylanase and endocellulase production by *Geobacillus* sp. WSUCF1 and *Cellulomonas* spp., respectively [42,43]. In general, *S. albidoflavus* strain SAMRC-UFH5 showed alkaline tolerance in the production of cellulase and xylanase, as suggested by the result of this study.

4.2. Effect of Incubation Temperature on Xylanase and Cellulase Production

Incubation temperature is paramount for optimal enzyme production due to alterations in microbial protein structure and properties with temperature variations [44]. At temperatures below or above the optimum, metabolic activities are reduced, with consequent inhibition in growth and enzymes synthesis [11]. While some studies suggest higher xylanase production with increased temperature, others have suggested otherwise [45,46]. In concordance with this study however, optimal xylanase production at 35 °C by *Streptomyces* sp. F2621 has been documented [38], while another study reported 32 °C as optimum with significant reduction of over 50% xylanase productivity by *S. halstedii* at 45 °C [45]. Previous studies have reported optimum temperatures of 30 and 26 °C for cellulase production by two *Streptomyces* strains and *B. subtilis* respectively [47,48]. In addition, Jaradat et al. [49] also reported optimal temperature of 30 °C for cellulase production by *Streptomyces* sp. strain J2. Comparatively, a higher temperature of 50 °C for production of CMCase by *B. licheniformis* MVS1 and *Bacillus* sp. MVS3 has been reported [35]. Differences in optimal temperature for enzyme production could be attributed to strain variations between bacterial groups and adaptability to temperature dynamics [50].

4.3. Effect of Agitation on Cellulase and Xylanase Production

The result of this study is in accordance with that of Techapun et al. [50], who reported agitation of 150 rpm as optimal for xylanase production by *Streptomyces* sp. Ab 106. On the contrary, Sepahy et al. [51] reported 200 rpm as optimal for xylanase production by *Bacillus mojavensis* AG137 cultured on agricultural waste. Whereas Saratale et al. [31] reported static condition as optimal for endocellulase production by *Streptomyces* sp. MDS using carboxymethyl cellulose as carbon source, this was not the case in this study as the least cellulase production was recorded at stationary condition (Figure 4b). An optimal agitation of 300 rpm was recorded for cellulase production by *Streptomyces* sp. T3-1, a transformant strain cultivated in 50 L fermenter [52]. Agitation affects the rate of oxygen mass transfer, and is crucial for maximal production in microbial enzyme fermentation [52]. However, high agitation speed may also result in high shear stress and impact negatively on mycelia growth [53,54]. In this study, low agitation speed seemed favorable for cellulase and xylanase production.

4.4. Time Course and Kinetics for Production of Cellulase and Xylanase

The decline in cellulase production observed in this study at 72 and 84 h of incubation could be attributed to nutrient depletion, proteolysis, or both [55], while the further peak observed at 96 h could be due to some quantity of cellulase released from aging microbial cells undergoing autolysis [51]. Different optimal incubation times for cellulase and xylanase production by other bacterial strains have been documented [31,41,56]. Literature reports from previous studies reveal that the incubation time for optimal cellulase production by other *Streptomyces* species were in the range of 72–196 h [28,47,56,57]. Nevertheless, the findings of this study agree with that of other authors who reported 48 h as optimum for xylanase production [58–60]. However, most other studies recorded longer optimum incubation time for both cellulase and xylanase production using different substrates [27,36,45,61].

4.5. Effect of Carbon Source Inducers on Cellulase and Xylanase Production

Cellulases and xylanases are members of glycoside hydrolases, which are well-known for their side activities (not investigated here), for instance, endoglucanases are capable of degrading hemicellulose at a low level and some xylanases exhibit cellulolytic activity. This has been attributed to their modular structure comprising a noncatalytic carbohydrate binding module (CBM) and the catalytic domain [62]. Studies have shown that the array of enzymes produced by microbes is dependent on the type of substrate on which they are cultured [63]. Nevertheless, the result of this study agrees with an earlier report on inducing effect of saccharides for hemi(cellulases) production by a *Streptomyces* sp. [29]. The mechanisms governing extracellular enzyme production in response to carbon sources are said to be influenced by low-molecular-weight fragments of substrates produced by a small quantity of constitutively formed enzymes [64]. Various reports show that cellulase and xylanase induction seems to be species-specific in different bacterial strains. For instance, studies have reported that cellulase production by *Bacillus* VITRKHB was optimal in fermentation media supplemented with xylose as the carbon source; on the other hand, the best carbon source for cellulase production by *B. subtilis* and *B. pumilus* EWBCM1 was lactose and galactose, respectively [48,65,66]. Furthermore, xylose was the preferred carbon for xylanase production by *Arthrobacter* sp. MTCC 5214 [67], while Bhosale et al. [68] reported dextrose as the best inducer of xylanase production by *Streptomyces rameus*. The *Streptomyces* strain in this study was able to produce cellulase-free xylanase as well as xylanase with low cellulase activity, depending on the carbon source (Table 1). Whereas the extracellular enzyme produced by the bacterial strain in the absence of carbon source (control medium) had low activity of xylanase, cellulase activity was not detected, which suggest that xylanase and cellulase production by *S albidoflavus* strain SAMRC-UFH5 was inducible [7]. In addition, the highest xylanase activity recorded in wheat straw signifies the versatility of this strain for xylanase production using low cost carbon source.

5. Conclusions

Optimal conditions for xylanase and cellulase production were investigated by measuring xylanase and cellulase activity at the varied incubation conditions. Classical optimization for xylanase and cellulase production by *S. albidoflavus* strain SAMRC-UFH5 achieved an 84.27- and 45-fold increase respectively. Production was favored by alkaline pH, mesophilic temperature, mild agitation speed, and a relatively short incubation time. Furthermore, the strain was able to produce higher amounts of xylanase than cellulase, using both synthetic and lignocellulosic wastes carbon sources. This implies that the strain could serve as resource for low cost production of cellulase-free xylanase of importance in different industrial processes including biopulping, food and feed production.

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