



(RESEARCH ARTICLE)



Isolation and optimization of the fermentation condition of cellulolytic microbial isolates from cassava waste water

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Abstract

The study was conducted to isolate and identify cellulose producing microorganisms from cassava waste water. Cassava waste water sample was obtained from a cassava processing factory at Lokogoma town in Wushishi Local government area of Niger State. The microorganisms were isolated, identified and counted by standard microbiological methods. The mean bacteria count ranges from 6.8×10^3 cfu/mL⁻¹ to 2.1×10^3 cfu/mL⁻¹ while the fungi count ranges from 3.2×10^3 cfu/mL⁻¹ to 1.2×10^3 cfu/mL⁻¹. A total of eight (8) bacterial; *Staphylococcus aureus*, *Bacillus anthrax*, *Bacillus subtilis*, *Escherichia coli*, *Klebsilla sp*, *Bacillus megaterus*, *Staph. Epidermidis* and *Pseudomonas aeruginosa*, and six (6) fungi; *Saccharomyce serivicea*, *Aspergillus niger*, *Penecillium sp.*, *Muccor sp.*, *Aspergilus flavus* and *Aspergilus fumigetis* isolates were identified in the waste water. Among these organism, the best cellulase activity was recorded for *Bacillus subtilis* (10.39×10^{-4} mg/ml/sec) and *Aspergillus niger* (11.21×10^{-4} mg/ml/sec). However, maximum activity was obtained at pH ranges from 3 ~ 9, temperature ranges from 30 °C ~ 80°C and substrate concentrations ranges from 1.5% ~3.0%. In conclusion, cassava processing water regarded as waste water could be an alternative source of microorganisms capable of producing cellulase enzyme for industrial purposes.

Keywords: Cassava waste water; Microbial isolate; Cellulase; Optimization

1. Introduction

Biomolecules derived from natural resources are playing a major role in manufacturing products needed for daily use [1]. Enzymes are one of those molecules that are globally recognized for their multifarious applications in industries. For instance, their utility in brewing, dairy products, detergents, food and feed, pharmaceutical production, and paper and pulp industry is huge. One of those most widely used enzymes is cellulase [2]. According to recent global cellulase market analysis reports, the demand for this enzyme is exponentially increasing.

Cellulose the substrate of cellulase is the most abundant biological compound on terrestrial and aquatic ecosystem, and is the main component of plant biomass [3]. It is the dominant waste material from agricultural industry in the form of stalks, stems and husk, and there has been great interest in utilizing cellulose as an energy resource and feed [4]. The cellulose is composed of D-glucose units linked together to form linear chain via β -1, 4-glycosidic linkages [5]. Cellulase catalyses the hydrolysis of cellulose, it is composed of endoglucanase and exoglucanases including cellobiohydrolases and β -glucosidase. The enzyme breaks β -1,4-linkages in cellulose polymer to release sugar subunits such as glucose. This notion is applied in industries either cellulose is utilized as a raw material or cellulose degradation is a must [6].

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Fungi, bacteria, and actinomycetes are recorded to be efficient cellulase enzyme producers in natural environment. These microorganisms must secrete cellulases that are either free or cell surface bound. Their enzyme production efficiency and the enzyme complex composition are always diverse from each other. Although both aerobic and anaerobic microorganisms produce these enzymes, aerobic cellulolytic fungi, viz., *Trichoderma viride* and *T. reesei* are excessively studied.

Cassava (*Manihot esculanta*) is a root tuber crop that is widely cultivated in the tropical regions of the world [7], as a shrubby perennial crop that grow to a height of 6-8 ft. Today, Nigeria is the biggest producer of cassava after Brazil and Thailand [8]. During the processing of cassava tubers in various products, liquid waste waters generated was reported to cause serious havoc to vegetation, houses and bring about infection. In addition microbial enzymes have extensively been used in most of industrial processes and has led to high demand and cost of these enzymes. To this end, we aimed to isolate cellulolytic bacterial and fungi from cassava waste water and also to determine their optimum condition for efficient cellulolytic activity.

2. Material and methods

2.1. Sample collection and processing

Cassava waste water was obtained from a cassava processing factories located at Lokogoma village under Wushishi Local Government Area, Niger State. The samples were collected using swap sticks. The swap sticks were placed on a clean and tight container and transported to the microbiology laboratory of the Federal University of Technology Minna in an ice bags for analysis. Stock samples were maintained at 4°C in the refrigerator. The chemicals used were analytical grade, while the water was glass distilled.

2.2. Isolation and identification of Associated Microorganisms

The associated microorganisms of the samples were isolated rising serial dilution pour plate. In this case, 0.1 mL of dilutions $\times 10^{-5}$ and $\times 10^{-2}$ of the samples were inoculated on nutrient agar (NA) and potato dextrose agar (PDA) for bacteria and fungi, respectively. The inoculated media were incubated at 37°C for 24 h for bacteria and at 30°C for 3-5 days for fungi. Discrete colonies that developed on the plates were counted and recorded as colony forming unit per milliliter (cfu mL⁻¹). The pure cultures of bacteria obtained by sub culturing were identified using the methods of Holt et al. [9], while the fungal isolates were identified by Barnett and Hunter [10]. The bacterial isolates were characterize using catalase, oxidase, motility indole urea methyl red, voges-proskauer, starch hydrolysis, and sugar fermentation test.

2.3. Production and Optimization of cellulase from microbial isolates.

The fungal and bacterial isolates were screened for cellulase activity according to the method The method described by Cowan and Steel [11]. The effect of temperature on microbial cellulase activities was assayed at 20-80°C, pH 6.9 for 30 min. After a 10 min incubation, cellulase activity was determined for each temperature regime. The effect of pH on cellulase production was determined on starch solutions (1% starch and 0.006 M NaCl) at pH 3.0 – 9.0, 30°C for 30 min. The cellulase activity was determined as earlier outlined. Cellulase activities of the various crude preparations were determined at various substrate concentrations of 1-9% starch solutions containing 0.006 M NaCl in 0.2 M phosphate buffer at pH 6.9 by the method earlier described.

2.4. Data analysis

All analysis was conducted in triplicate. Data were analyzed using statistical package for social science (SPSS) and presented as man value.

3. Results

3.1. Frequency of Microbial isolates from cassava waste water

The mean counts of bacterial and fungal isolates from cassava waste water were 3.88×10^3 cfu mL⁻¹ and 2.23×10^3 cfu mL⁻¹ respectively. *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterus* and *Klebsilla sp.* had higher percentage bacterial occurrence of 58.33%, 50.00%, 25% and 25% respectively while (8.33%) *Staph. Epidermidis* had the least. The higher percentage occurred fungal isolates were *Saccharomyce servicea* (83.33%), *Aspergillus niger* (66.66%) and *Penecillium sp.* (50.00%).

Table 1 Frequency of Microbial isolates from cassava waste water.

Bacterial Isolates		Fungi isolates	
Bacteria	Occurrence (%)	Fungi	Occurrence (%)
<i>Staphylococcus aureus</i>	7 (58.33%)	<i>Saccharomyce servicea</i>	10 (83.33%)
<i>Bacillus anthrax</i>	2 (16.66 %)	<i>Aspergillus niger</i>	8 (66.66%)
<i>Bacillus subtilis</i>	6 (50.00%)	<i>Penecillium sp.</i>	6 (50.00%)
<i>Escherichia coli</i>	2 (16.66%)	<i>Muccor sp.</i>	4 (33.33%)
<i>Klebsilla sp.</i>	3 (25.00%)	<i>Aspergillus flavus</i>	3 (25.00%)
<i>Bacillus megaterus</i>	3 (25.00%)	<i>Aspergillus fumigetus</i>	1(8.33%)
<i>Staph. Epidermidis</i>	1 (8.33 %)		
<i>Pseudomonas aeruginosa</i>	2 (16.66%)		

Key: cfu mL⁻¹ = colony forming unit per millimeter Key: + = Present; - = Absent

3.2. Cellulase activity of microbial isolates

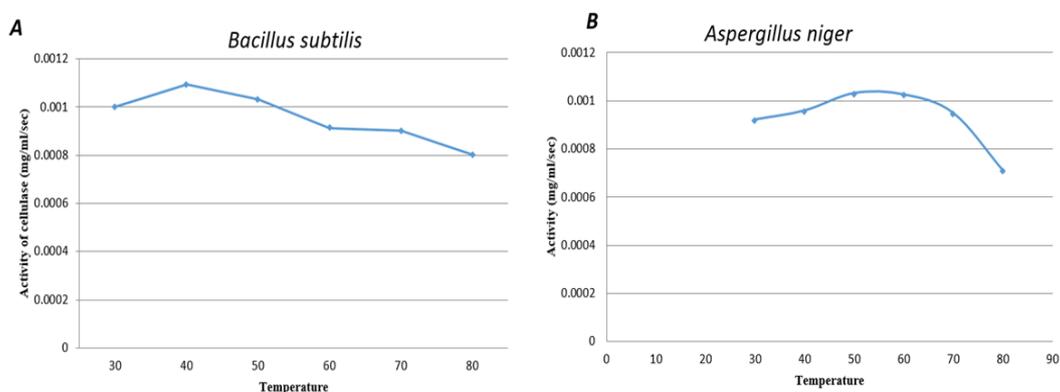
Among the 5 bacterial isolates tested for cellulose activity, *Bacillus subtilis*, *Bacillus anthracis* and *Bacillus megaterium* having cellulase activity 10.39×10^{-4} (mg/ml/sec), 8.99×10^{-4} (mg/ml/sec) and 8.61×10^{-4} (mg/ml/sec) respectively were the most potent cellulase producing isolates, while *Aspergillus niger*, *Aspergillus fumigatus* and *Pencillium sp.* having cellulase activity 11.21×10^{-4} (mg/ml/sec), 7.54×10^{-4} (mg/ml/sec) and 6.53×10^{-4} (mg/ml/sec) respectively were the most potent cellulase producing fungi isolates (Table 1)

Table 2 Cellulase Activity of bacterial and fungal isolates

Bacteria	Activity (mg/ml/sec)	Fungi	Activity (mg/ml/sec)
<i>Bacillus anthracis</i>	8.61×10^{-4}	<i>Aspergillus niger</i>	11.21×10^{-4}
<i>P. aerogenosa</i>	5.58×10^{-4}	<i>Aspergillus fumigatus</i>	7.54×10^{-4}
<i>Klebsilla sp.</i>	3.58×10^{-4}	<i>Aspergillus flavus</i>	6.38×10^{-4}
<i>Bacillus subtilis</i>	10.39×10^{-4}	<i>Penicillium sp.</i>	6.53×10^{-4}
<i>Bacillus megaterium</i>	8.99×10^{-4}		

3.3. Optimization of temperature for microbial cellulase activity

The maximum cellulase activity (10.01×10^{-4} and 10.01×10^{-4}) by *Bacillus subtilis* were observed at temperature range of between 40°C and 30°C while the optimum temperature for cellulase activity by *Aspergillus niger* were observed at 50, 60, 40 °C having cellulase activity of 10.25×10^{-4} , 10.31×10^{-4} and 9.59×10^{-4} respectively (figure 1).

**Figure 1** Optimization of temperature for cellulase production by microbial isolate form cassava waste water

3.4. Optimization of pH for microbial cellulase production

The maximum cellulase productions (9.95×10^{-4} , 9.32×10^{-4} and 9.19×10^{-4}) by *Bacillus subtilis* were observed at pH 6, 7, and 8 while the optimum cellulase production by *Aspergillus niger* were observed at pH 6, 7 and 8 having cellulase activity of 11.93×10^{-4} , 11.25×10^{-4} and 10.30×10^{-4} respectively (figure 2).

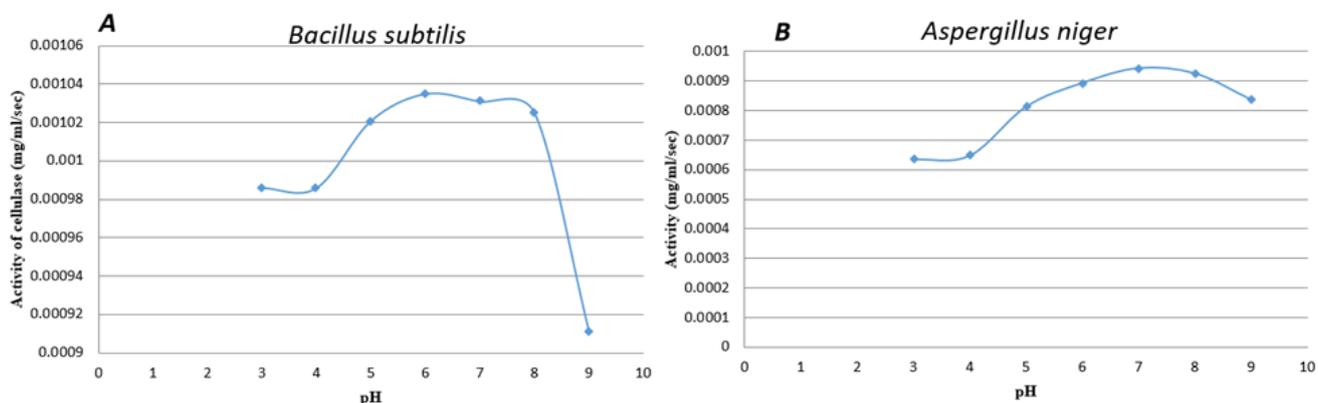


Figure 2 Optimization of pH for cellulase production by microbial isolate form cassava waste water

3.5. Optimization of substrate for microbial cellulase production

The maximum cellulase production of 15.31×10^{-4} , 15.20×10^{-4} , 12.18×10^{-4} by *Bacillus subtilis* was observed at substrate concentrations of 2.5%, 3.0% and 1.5% respectively while the optimum substrate concentration for cellulase production by *Aspergillus niger* were observed at 3.0%, 3.5% and 4.0% substrate having cellulase activity of 19.30×10^{-4} , 19.20×10^{-4} and 19.05×10^{-4} respectively (figure 3).

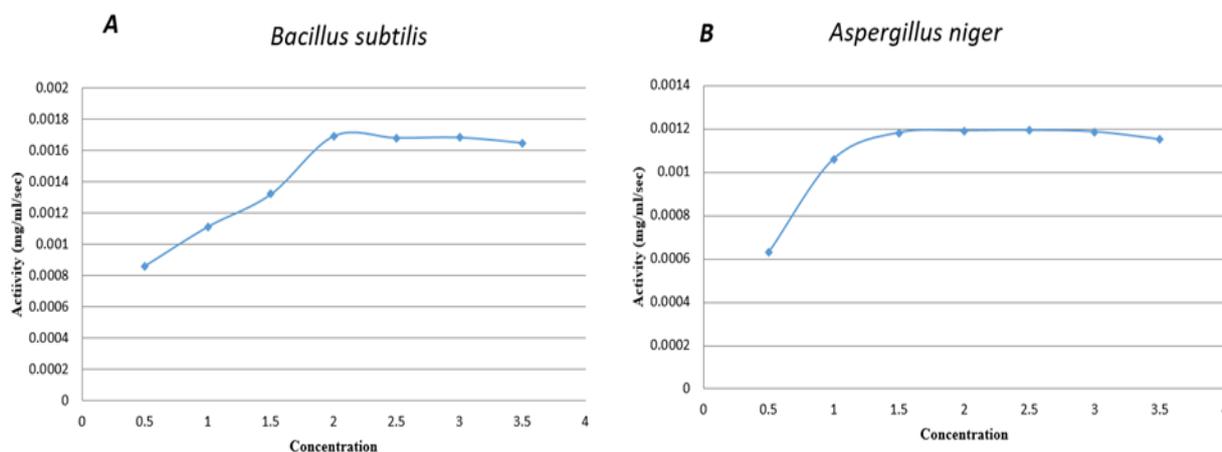


Figure 3 Optimization of substrate concentrations for cellulase production by microbial isolate form cassava waste water

4. Discussion

The successful isolation and identification of the diverse group of bacteria and fungi from the cassava waste water analyzed is in accordance with research cited from the literature that both bacteria and fungi are associated with cassava waste water. However, most of these microbial isolates have been implicated during the processing of cassava tubers into various products [12]. These microbial isolates may probably have originated from soil, water and materials used during the processing of cassava, while the variations of the isolates may be due to the handling process and the prevailing environmental conditions [13]. The high microbial counts from the cassava waste water analyzed may be due to lack of efficient control measures in discharge of waste water into the environment. Uzochukwu et al. (2001) reported that high level of cassava waste water are produced daily and drained onto roads, streets, rivers and

agricultural lands in gari producing communities of Nigeria. These singular activities tend to expose the waste water to microbial contamination [14]

Though the statement by Korpole [3], that cellulose is the most abundant biological compound on terrestrial and aquatic ecosystem and is the main component of plant biomass could also be attributed to the findings of the current research work. A similar statement from Deke *et al.* [4], that the dominant waste material from agricultural industry in the form of stalks, stems and husk, there has been great interest in utilizing cellulose as an energy resource and feed.

From the current findings to have recorded a high microbial counts of 7.2×10^3 and 6.3×10^3 is likely in accordance with the work of Arotupin [8] to have recorded 8.02×10^5 and 5.00×10^2 for both bacteria and fungi respectively. Microbial isolates of cassava waste water consisted of *Bacillus subtilis*, *Bacillus lichinoforms*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus lichinoforms*, *Lactobacillus bulgaricus*, *Streptococcus feacalis*, *Klebsiella pnemonea*, *E. coli*, *Bacillus subtilis*, *Bacillus lichinoforms*, *Micrococcus luteus* bacterial while *Saccharomyce servicea*, *Aspergillus niger*, *Penecillium sp.*, *Muccor sp.*, *Aspergillus flavus* and *Aspergillus fumigetus* for fungal isolates. Arotupin [8] analyzed similar microorganisms although not all which includes bacterial isolates *Aerococcus viridens*, *Bacillus subtilis*, *Bacillus sp.* *Corynebacterium manihot* and *Lactobacillus acidophilus* while fungal isolates include; *Aspergillus fumigatus*, *A. niger*, *A. repens*, *Articulospora inflate* and *Geotrichum candidum*.

The microbial isolates screened for cellulase production from the current findings includes, *Bacillus anthracis*, *Bacillus subtilis*, *Klebsiella sp.*, *Bacillus megatarium*, *Pseudomonas aeriginosa*, *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Penecillium sp.*, recording a potent activity in *Bacillus subtilis* (10.39×10^{-4}), *Bacillus megaterium* (8.99×10^{-4}) and *Bacillus anthracis* (8.61×10^{-4}) for bacterial isolates while the 3 potent isolates for fungal include *Aspergillus niger* (10.39×10^{-4}), *Aspergillus fumigatus* (7.54×10^{-4}) and *Penicillium sp.* (6.53×10^{-4}) respectively. Recording a positive activity from *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus fumigatus* is in accordance with the report of Arotupin [8] to have reported that all the microbial isolates were screened for production of cellulase, *Aerococcus viridens*, *Bacillus sp.* *Corynebacterium manihot*, *A. niger*, *Articulospora inflate*, *Geotrichum candidum* and *Candida utilis* were positive for cellulase. *Bacillus subtilis*, *Lactobacillus acidophilus* and while *A. fumigatus* and *A. repens* were positive for cellulase.

From the current finding to have screened *Penicillium sp.* and *Aspergillus niger* to be positive in cellulase activity is in agreement with work of Saravanan *et al.* [15] studied production of cellulose using *Trichoderma reesei* in solid state fermentation. Some species of *Penicillium* i.e. *Penicillium iriensis* and *P. citriviride* produce significant quantities of cellulase, when grown under different conditions. A similar report from Peig *et al.* [16] stated that fungal genera like *Aspergillus* and *Trichoderma* are taught to be cellulase producers and crude enzymes produced by these microorganisms are commercially available for agricultural.

The highest activities of cellulose production from *Bacillus subtilis* and *Aspergillus niger* from the current research is in conformity with reports of Xia and Cen [17] that *Bacillus subtilis* CBTK 106 can produce a considerable amount of cellulase activity. In another similar report Riley, [18] studied production of cellulose using *Bacillus subtilis* CEL PTK 1 from cow dung. Hebeish and Ibrahim [19] studied cellulase productivity by *Bacillus subtilis* KO strain using CMC zone and dinitro salicylic acid. *Aspergillus niger* produces highly active cellulase when grown in liquid media by both surface and submerged culture methods and recently by solid state fermentation [20]. Omajasola *et al.* [21] studied production of cellulase from the orange peel using *Trichoderma longibrachiatum*, *Aspergillus niger*.

In addition, from the present research recording copious activity of cellulase from *Bacillus subtilis*, *Bacillus megaterium* and *Aspergillus niger* is in accord with work of Oboh [22] to have reported higher activities in *Bacillus*, *Aspergillus*, *Cadida* and *Saccharomyces* for the production of cellulase. Cellulase is one of the most useful enzymes in industry. Cellulase can be produced by fungi, bacteria or actinomycetes, but the most common producer is fungi [23].

5. Conclusion

A total number of 14 isolates were obtained from the cassava waste water, the isolates includes *Staphylococcus aureus*, *Bacillus anthrax*, *Bacillus subtilis*, *Escherichia coli*, *Klebsilla sp.*, *Bacillus megaterus*, *Pseudomonas aeroginosa*, *Staph. Epidermidis*, *Saccharomyce servicea*, fungi include *Aspergillus niger*, *Penecillium sp.*, *Muccor sp.*, *Aspergillus flavus* and *Aspergillus fumigetus*. The microorganism which appear to be the best promising in cellulose production includes *Bacillus anthracis*, *Bacillus subtilis*, *Klebsiella sp.*, *Bacillus megatarium*, *Pseudomonas aeriginosa* while fungi isolates includes *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus* and *Penecillium sp.* but fungi produce cellulose best when compare to the bacteria isolates. These enzymes have direct applications in industrial processes.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exists

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