



UTILIZATION OF AGROWASTES FOR ENHANCED PRODUCTION OF CELLULASE FROM INDIGENOUSLY ISOLATED MICROORGANISM

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Abstract: A homo polymer of anhydrous glucose, cellulose is widely found in nature and is made up of glucose residues joined in a β -1, 4 fashions. Important sources of cellulose include bacteria, algae, and plants. Moreover, lyocell, rayon, and modal are made of synthetic or regenerated cellulose. Agricultural wastes like orange and banana peels, sugarcane bagasse, wheat straw, oat hulls, corn bran and peanut shells contain cellulose. Food, personal care items, clinical bioscience, bioactive compound delivery, the pharmaceutical industry, paper manufacturing, wastewater treatment, and sensor applications are all areas that use cellulose. Cellulases are enzymes that break down cellulose into sugar so that it can be bioprocessed into ethanol. Cellulases were produced by a broad range of bacteria and fungus. The use of agrowastes to increase cellulase production using specific microbial isolates was the main focus of this study. This study's goal is to use submerged fermentation to create bacterial and fungal cellulases from agricultural waste.

Key words: Agrowaste, Cellulase, *Bacillus licheniformis*, Carboxymethyl Cellulose (CMC), pollution

INTRODUCTION

Cellulose is found naturally as a polysaccharide formed by repeating monomeric units, i.e., β -D- glucopyranose linked together by a bond between C1 and C4 carbon atoms. The cellulose chain consists of D-glucose units, which have a reducing end and a non- reducing end (Klemm *et al.* 2005). There are different forms of cellulose with varying mechanical and pharmaceutical properties available in the market. The classification is based on the level of crystallinity, shape, and size of the particles. Cellulose had been categorized into three distinctive types including powdered cellulose (PC), microcrystalline cellulose, and low crystallinity powdered cellulose (LCPC). Microcrystalline cellulose has pharmaceutical importance and is the most available form. It is further processed with particular chemicals techniques or with certain compounds as silicon dioxide to generate MCC type II or silicified MCC (Shokri & Adibkia, 2013). Cellulose-rich plants are wood, jute, hemp, sisal, and ramie. Cellulose makes up 90% of cotton (Lavanya *et al.*, 2011). Cellulose has been isolated from fruit peels such as orange peels, banana peels, and pomelo. Agricultural wastes such as, oat hulls, corn bran, soybean hulls, wheat straw, dried beet pulp, and pea hulls, are cellulose-rich sources (Sundarraj & Ranganathan, 2018). Chemical processing of natural cellulose results in the production of synthetic fibers like rayon, modal, and lyocell. These are commonly known as regenerated cellulose (Lavanya *et al.* 2011). Bacterial cellulose is used in food, personal care products, bioactive compound delivery, and clinical bioscience due to its unique properties. BC has high purity, diverse forms

and shapes, and an easy production process, and its ability to absorb color and flavors from culture media makes it important for use in the food manufacturing sector (Ullah *et al.* 2016). Dispersed system (suspension and emulsion) and therapeutic solution are most widely thickened with cellulose derivatives (Shokri & Adibkia, 2013). Cellulases are cellulose-degrading enzymes that break down cellulose into sugar for bioprocessing into ethanol. Cellulases are a group of hydrolytic enzymes that cleave the beta 1, 4-glycosidic bond in cellulose (Sulyman *et al.* 2020). A diversity of microorganisms, including bacteria and fungi oxygen-dependent or independent microorganisms, temperate microorganisms, and heat-loving bacteria, produce cellulases that break down cellulose (Acharya *et al.*, 2008). Cellulases are classified into three main categories comprising endoglucanases, exoglucanases, and beta-glucosidase. To produce cellulases, solid-state fermentation (SSF) and submerged fermentation (SmF) are the two industrial processes. For large-scale production, due to the availability of sophisticated machinery and more control over environmental factors, the latter process is frequently employed. On an industrial scale, SSF processes have yet to replace SmF processes, despite being in use for several decades, especially in the eastern region of the world (Gautam *et al.* 2011). Cellulases are used in various industries including textile and detergent, pharmaceutical sector, agriculture sector, bioethanol production and beverage industry. For restriction of plant diseases and improving crops, cellulases have been widely used in agriculture along with pectinases and hemicellulases. Phytopathogens cell walls are broken by B-glucanases isolated.

Utilization of Agrowastes for enhanced production of cellulase from indigenously isolated microorganism

MATERIALS AND METHODS

Sample collection

Three agricultural wastes have been taken, i.e., sugarcane bagasse, wheat straw, and peanut shells to find out is the best agricultural waste for the production of cellulase from different microbial strains. This procedure includes media preparation, sterilization, inoculum preparation, incubation, and enzyme assay.

Media preparation

Three Fermentation broth having peanut shells, sugarcane bagasse, and wheat straw (0.5g) as substrate sources were prepared as flask A, flask B and flask C separately. In all flasks yeast extract (0.05g), K_2HPO_4 (0.05g) and $MgSO_4$ (0.05g) in 50 ml distilled water. Ingredients were thoroughly mixed with the help of stirrer and final volume makeup to 100 ml with distilled water.

Inoculum preparation and incubation

Fermentation media 5.0 and 45 ml were prepared in three test tubes and flask respectively. The test tubes and flasks were autoclaved at 121°C for 15 minutes to avoid contamination. After sterilization, *Aspergillus niger* in test tubes A, *Penicillium* in test tube B and *Bacillus licheniformis* EP3 in test tube C were streaked and test tube A and B were incubated for 3 days at 30°C temperature and tube C at 37°C for 24 hours temperature in separate incubators. After incubation, each inoculum was transferred to their respective flask and Flasks A and B were incubated at 30°C for 5 days, and flask C at 37°C for 24 hours separately.

Media and inoculum preparation

Peptone, yeast extract, K_2HPO_4 , and $MgSO_4$ are required for CMC (carboxymethyl cellulose) media. 100 ml of distilled water were used to dissolve 1.0 gm of CMC, peptone, and yeast extract, as well as 0.1 gm of K_2HPO_4 and $MgSO_4$. To create the inoculum, three test tubes were designated A, B, and C. From each flask 2.0 ml were used to prepare the inoculum. These tubes and flasks were autoclave for 15 minutes at 121°C to prevent contamination. *Aspergillus niger* was cultivated in test tube A, *Penicillium* and *Bacillus licheniformis* were introduced to test tubes B and C, respectively, while. Test tubes A and B were maintained at 30°C for three days in an incubator, while test tube C was maintained at 37°C for 24 hours. Each inoculum was transferred to its respective fermentation flask after the specific incubation period. Following transfer, incubate the bacterial and fungal strains in flasks for 24 hours at 37°C and 05 days at 30°C, respectively

Effect of pH on cellulase production

Media with varying pH values from 6.0-8.0 were prepared in order to determine the ideal pH for maximum enzyme production, and culture were then incubated for different time and temperature according to their growth requirements. Three media were prepared having different pH values ranging from 6.00-8.00. Media were prepared using sugarcane bagasse (0.5 gm), $MgSO_4$ (0.05 gm), K_2HPO_4 (0.05 gm) and yeast extract (0.5 gm). Three flasks, designated A, B, and C, each contain 50 ml of media and pH was adjusted to 6.00, 7.00 and 8.00 using 1.0 N NaOH or 1.0 M HCl. Media flask was inoculated

with *Bacillus licheniformis* EP3 and incubate for 24 hrs at 37°C. The *Bacillus licheniformis* EP3 was inoculated into the chosen medium and incubated 12, 24 and 48 hours in order to identify the peak of cellular growth and the production of cellulase

Enzyme Assay

For enzyme assay, substrate was prepared using 0.2 gm. cellulose dissolved in 100 ml citrate buffer (pH 6.5). Take 1.0 ml substrate and 0.1 ml of CFF in a three separate test tubes and kept at 50°C for 15 minutes. To each test tube 1.0 ml DNS was added and kept in boiling water bath for 5.0 minutes. For blank, 1.0 ml distilled water and 1.0 ml DNS in a test tube was added and same procedure was adopted as mentioned for test. Optical density was taken on UV-visible spectrophotometer after adding 4.5 ml distilled water.

Enzyme Units:

A cellulase unit is defined as “the amount of enzyme that liberates 1.0 μ mol of reducing sugar from carboxymethyl cellulose in one minute at 37 °C”.

RESULTS AND DISCUSSION

Three substrate peanut shell, sugarcane bagasse and wheat straw were used for the production of cellulase.

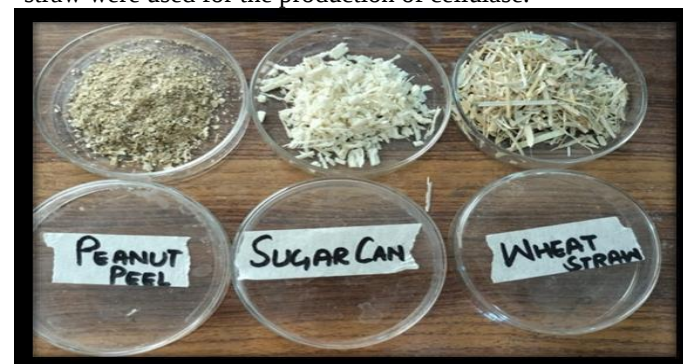


Fig 1: Peanut shells (A), Sugarcane bagasse (B), Wheat straw (C)

The most remarkable discovery was that the presence of the lignocellulose biomass (sugarcane bagasse) appeared to induce the production of xylanases and endoglucanases during sequential cultivation. This was because the fungus first produced xylanases to break down the hemicellulosic fraction, and then it produced endoglucanase to convert cellulose into fermentable sugars. (Cunha *et al.* 2012).

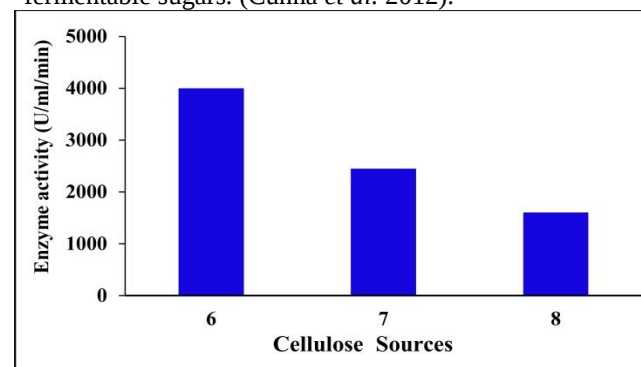


Fig 2: Production of cellulase from three substrate

Utilization of Agrowastes for enhanced production of cellulase from indigenously isolated microorganism

Production of cellulase from microbial strains

The results indicated that *Bacillus licheniformis* EP3 showed the highest enzyme activity as compared to *Aspergillus niger* and *Penicillium*. The highest enzymatic efficiency and cellulolytic potential make EP3 the best strain for cellulase production.



Fig 3: Flask showing microbial growth

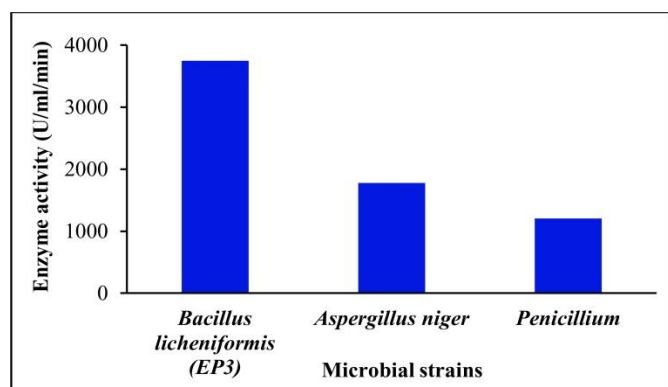


Fig 4: Cellulase production from different microbes

According to a report by Ghani *et al.* (2013), *B. licheniformis* has a high potential for producing large quantities of different extracellular enzymes with significant commercial value. Cellulases, also referred to as avicel-hydrolyzing enzymes (avicelases), were produced by *Bacillus* sp. SMIA-2 when it grew in a liquid medium containing sugarcane bagasse as a carbon source (Ladeira *et al.* 2015).

Effect of pH on cellulase production

A medium pH is essential for fermentation's role in the production of enzymes. Enzyme production, metabolic activity, and microbial growth are all impacted by the pH of the medium. Each microbe has a preferred pH range, and changes to this range affect microbial growth, enzyme synthesis, and metabolic activity. In the present study, maintaining the initial pH of the medium at 6.00 before sterilization resulted in the highest level of enzyme production (Figure 5). The findings demonstrated that slightly acidic conditions are more conducive to the production of cellulase,

with *Bacillus licheniformis* EP3 exhibiting the highest cellulase activity at pH 6.0. However, as indicated by their enzyme activity, cellulase production declined at alkaline pH. A report claims that the pH of the medium affects the makeup of the cell walls of microorganisms (Ellwood and Tempest, 1972).

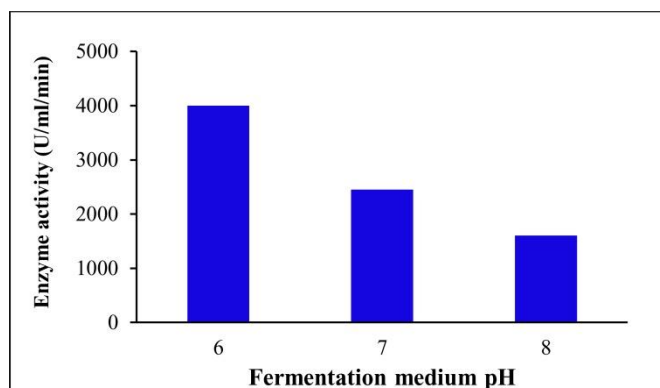


Fig 5: Effect of media pH on cellulase production

Effect of time on cellulase production

For optimal enzyme production, the bacterial culture was cultivated in the fermentation medium for varying durations, i.e. 12, 24 and 48 hours (Figure 6). Cell growth and enzyme production were found to be linearly related, with the highest levels of cellulase and cell growth occurring after 24 hours. Additionally, it was discovered that cellular growth began to slow down after 24 hours, which might be due to the fact that bacteria are susceptible to metabolite repressions that occur during growth (Cordeiro *et al.* 2002). It was reported that maximum production of CMCase by *Bacillus licheniformis* BCLLN-01 was found in 72 hours. Researchers also found that bacterial strains needed the same amount of time to grow to their maximum capacity and produce amylase (Bajpai and Bajpai, 1989; Saito and Yamamoto, 1975).

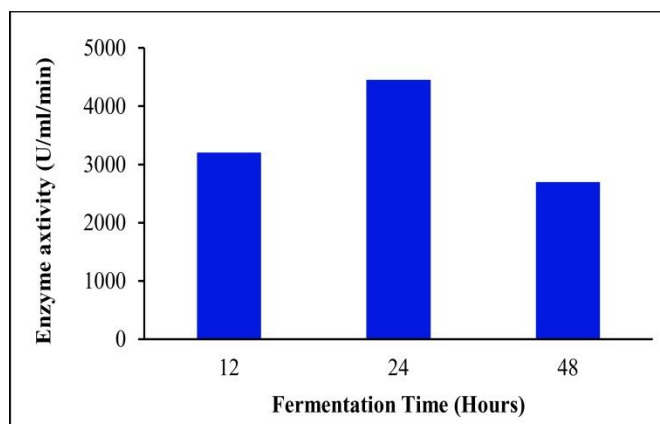


Fig 7: Effect of time on cellulase production

CONCLUSIONS

This study concentrated on using agrowastes, including sugarcane bagasse, peanut shells, and wheat straw, to produce cellulase. Due to its high cellulose and hemicellulose content,

Utilization of Agrowastes for enhanced production of cellulase from indigenously isolated microorganism

sugarcane bagasse is the ideal source for the production of cellulase. This study also suggested that the *Bacillus licheniformis* EP3 is the most effective strain for producing cellulase. This study supports environmentally friendly

biotechnological methods, lowers pollution and waste degradation and improves environmental sustainability.

Conflict of interest

Authors declare no conflict of interest.

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