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RECYCLING CORN COBS FOR IMMOBILIZATION OF CELLULASE PRODUCING BACTERIA

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Abstract: Corn cobs are agricultural waste refers to the residual material left after the kernels of corn have been harvested. This byproduct is often underutilized, despite its potential as a valuable resource. Corn cobs can be repurposed for various applications, including bedding, biofuel production, and as a raw material in the production of biodegradable plastics. In the present study, cellulase-producing gram negative bacteria was isolated from soil and screened for cellulase activity by congored assay. Corn cobs were used as a support material for the immobilization of cellulase-producing organisms, followed by the determination of cellulase activity. Corn cobs were sun-dried and allowed for alkali pretreatment for 24 hours, and then dried again for one day. Twenty-five milliliters of CMC broth made up the immobilization medium. Cellulase-producing bacteria were then mixed with the alkali-treated corn cob and incubated for 24 hours. The following day, corn cobs immobilized with bacteria were collected and inoculated in CMC broth for cellulase activity and incubated at 37 °C for 24 hours. Following a 24-hour period, the media was gathered, centrifuged, and the supernatant was gathered and subjected to a DNS analysis for cellulase. It was discovered that corncobs were an excellent support material for immobilizing bacteria that produce cellulase.
Keywords: corncobs, cellulase, carboxy methyl cellulose, Congo red, Dinitro salicyclicacid, immobilization.

Introduction

Agro-waste are the sources of carbohydrates, which have intriguing morphological, chemical, and mechanical properties yet are frequently discarded or underutilized. Due to the scarcity of non-renewable resources, there is a need to valorize this waste as urbanization increases [1]. Corn cobs (*Zea mays*) are agricultural waste refers to the residual material left after the kernels of corn have been harvested. This byproduct is often underutilized, despite its potential as a valuable resource, and an enormous amount of this residue is produced annually; it is primarily made of cellulose and accounts for 80% of the waste materials in most communities. One of the most important structural elements of plant cell walls is cellulose, a polymer consisting of linear D-glucose molecules joined by 1,4-glycosidic bonds. Plant cell wall-degrading enzymes, are pectinases, xylanases, and cellulases. These are among the leading enzymes produced commercially for their diversified applications.

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Particularly, cellulases are responsible for cellulose degradation by hydrolyzing the β-1,4-glycosidic bonds [2]. β-glucosidase, endo-1,4-β-D-glucanase (endoglucanase), and exo-1,4-β-D-glucanase (exoglucanase) are the three enzymes that make up cellulase. Endoglucanase targets the internal sites of oligosaccharides present in carboxymethyl cellulose, cellooligosaccharides, or amorphous cellulose. In contrast, exoglucanase hydrolyzes the non-reducing ends of crystalline cellulose, resulting in the primary end products of cellobiose or glucose. Additionally, β-glucosidase acts on the non-reducing ends of both cellobiose and cellodextrin [3].

Cellulases can be obtained from solid or submerged fermentation of bacteria and fungi. The most common cellulolytic bacteria are Thermobifida fusca, Thermonospora sp., Streptomyces sp., Ruminococcus albus, Thermobispora bispora, Erwinia chrysanthemi, Clostridium sp., Cellulomonas sp., Bacillus sp., and Acetivibrio cellulolyticus [4]. The cellulolytic fungus is Trichoderma reesei. Cellulases play a vital role in various industries, including paper and pulp, textiles, and the animal feed industry, and are also used in bio refineries to hydrolyze biomass into simple sugars. Immobilization refers to technique which is used to fix or attach cells, enzymes, or other biological molecules to solid supports while maintaining their activity. Immobilization can done by various methods which includes Adsorption where the molecules are attached to a solid support through weak interactions (van der Waals forces). Covalent binding involves forming strong covalent bonds between the substance and the support. While in entrapment the biological molecules are enclosed within a matrix or gel, which allows substrates to diffuse in and products to diffuse out. Immobilized bio catalysts can be reused multiple times in reactions, reducing the overall cost of processes by minimizing the need for new materials. Immobilization facilitates the easy separation of enzymes or cells from reaction mixtures, simplifying product recovery and purification processes. Immobilized enzymes and cells often exhibit enhanced thermal and operational stability, allowing them to function under harsher conditions without denaturation. This study primarily involves immobilizing cellulase producing bacteria in pretreated corn cobs through adsorption and assessing its cellulase activity.

Materials and Methods:

Sample collection and isolation of bacteria:

Soil was collected from Madras Veterinary campus and weighed for 1g. The weighed soil was combined with 1 mL sterile distilled water. Further serial dilution was carried out to

make a dilution ranging from 10^1to10^7. Finally,200 µL of the aliquot from 10^5,10^6 dilutions was spread on nutrient agar to obtain pure culture.

Screening of Cellulase producing bacteria by Congo Red assay:

Isolated bacteria intended for screening of cellulase activity was analyzed through Congo red assay. The isolates were cultivated on 1% carboxy methyl cellulose media [5](0.2% K_2HPO_4, 1% CMC % agar, 0.03% $MgSO_4.7H_2O$, and 0.25% $(NH_4)_2SO_4$ at pH 7) [6] and incubated at a temperature of 30 °C for a duration of 15 minutes. They were treated with a Congo red solution at a concentration of 0.1% (w/v) [7]. After discarding the reagent solution, the plates were rinsed with a 1M NaCl solution for 10 minutes. The appearance of a clear zone around the colony serves as an indicator of cellulase enzyme activity [8]. Bacterial smear was prepared and heat fixed it and proceeded with staining, stain with crystal violet for 45 seconds, then by iodine for 45 seconds followed by decolourization for 10 seconds and add counter stain safarrin for 20 seconds and while examining under a microscope it was identified as gram-negative bacillus.

Inoculum preparation

Preparation of CMC broth containing g/l of CMC 3.0, KCl 2.0, MgSO4.7H20 20, Casmino acids 1.0, Yeast Extract 1.0, and Nacl 2.5M[9] in a conical flask and medium was autoclaved. A loop from pure culture was inoculated into the medium.

Pre treatment of corn cobs:

Locally procured corn cobs are cleaned and then chopped into bits. Followed by boiling for 5 mins, and these pieces were sun dried for one day. For the pretreatment phase, the corncob pieces were immersed in a 1% sodium hydroxide solution and maintained at 30 °C for 24 hours [10]. After this, the pieces were rinsed. Finally, the alkali-pretreated corncob pieces were again sun-dried.

Immobilization of corn cobs:

A 500 µl of overnight culture was introduced into 150 mL of CMC broth, which contains six pieces of alkali-treated corncobs, and the mixture was incubated at 30 °C for a duration of 48 hours. After 48 hours Corn cobs were removed from the CMC broth and introduced in to freshly prepared CMC broth without the inoculums and incubated for 48 hours. Enzyme activity was checked after 48 hours.

Enzyme Activity:

Preparation of crude enzyme:

Following the incubation period, the cultures were centrifuged, and the resulting supernatants were collected as the source of crude enzyme [11]. This crude enzyme solution was subsequently employed to assess enzyme activity.

DNS Method:

Cellulase activity was assessed using the DNS (3,5-dinitrosalicylic acid) method as described by Miller [12]. cellulase activity was determined by release of reducing sugar during hydrolysis of CMC by the enzyme. CMC dissolved in Tris–HCl for 10mL buffer at pH 7.0 used as substrate[13] . Prepared 1 ml of CMC solution was mixed with 1 ml of crude extract. The mixture was incubated for 30 minutes at 45 °C, after which the reaction was halted by the addition of DNS solution. The samples were then boiled for 10 minutes, cooled in water to stabilize the color, and the optical density was recorded at 550 nm[13].

The following formula was used to determine the cellulase activity.

Activity(U/mL)=reducing sugar(mg)*1000/Mm reducing sugar*30min*0.1mL

Reducing sugar content is the amount of reducing sugar produced (mg) and determined by the DNS method; reducing sugar Mm is simplified by using the molar mass of glucose (180 g/mol), incubation time is the cellulose hydrolysis time (30 min), and sample volume is the amount of crude extract analyzed (0.1 mL) [14].

1 unit is defined as the amount of enzyme required to release 1 μmol of reducing sugar per minute, under the test conditions [15].

Results and Discussions:

When soil sample was serially diluted and plated on nutrient agar, at 10^{-6} dilution only one colony was obtained which was subcultured and taken for further analysis. It was confirmed that the isolated organism from the soil was cellulase-producing bacteria through the Congo red assay, in which the organism showed zone of clearance in CMC agar plate. The bacteria was found to be gram-negative by Gram staining. Gram negative cellulose producing bacteria was immoblised on alkali-pretreated corn cobs and cellulose activity tested by DNS method was found to be 1.30 U/ml in the crude extract.

A study by Dos Santos et al.,[16] demonstrated that corn cob residual biomass is an inexpensive, effective, and promising material for laccases immobilization. The research showed that 74.8% of the immobilization yield was achieved using a 0.1 g/mL laccase solution, with a high recovered activity of 39.6%, comparing with immobilized cellulase

producing bacteria on corn cobs has shown cellulase activity ranging (1.30 U/ml) higher than the laccases immobilization. This resulted in a biocatalyst with an activity of 2123 U/kg. Maham Aftab et al[17] ,reported that *S. cerevisiae* MK-157 immobilized in corn cob matrix and produced the maximum xylanase (0.48 IU mL−1) when cultivated in Mineral salt medium, while in CMC broth immobilized cellulase producing bacteria on corn cobs showed increase in cellulase activity compared to xylanase immobilization .Zabin et al [18] reported Glutaraldehyde-activated calcium-alginate-immobilized purified xylanase showed recycling stability (87 %) up to seven cycles and the immobilized (crude) enzymatic hydrolysis of sweet sorghum bagasse resulted in the release of 8.1 g of reducing sugars per gram of bagasse after 48 hours, reducing sugars released from the hydrolysis of CMC by immobilized cellulase producing bacteria in corn cobs was lower(3.51 g) compared to hydrolysis of sweet sorghum bagasse. Therefore this study shows corn cobs are good at immobilizing and acts a excellent support material for immobilization

Conclusions:

Utilizing corn cobs offers a sustainable way to reduce agricultural waste. Agriculture waste corn cob was being produced at a high rate, and it can be used as a solid matrix to immobilize organisms. Corn cobs were used to immobilize the cellulase-producing bacteria detected by congo red, and the resultant cellulase activity was 1.30 U/mL. Corncobs were shown to be a great support material for immobilizing cellulase-producing bacteria. Enzyme-producing bacteria can be immobilized in corn cobs for industrial use to produce vast quantities of enzymes on a large scale while lowering production costs and waste

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