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Isolation, Characterization and Optimization Study of Cellulose Degrading Microorganisms and their Application in Ethanol production and Waste Water Treatment

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ABSTRACT
The cell wall of green plants contains an essential structural element called cellulose. With the aid of symbiotic microbes that dwell in their gut, some animals, particularly ruminants and termites, can digest cellulose. Microorganism-produced cellulase enzyme is crucial for the breakdown of cellulose. Bacteria having ability to produce cellulase have been identified in a various forms. In the current work, cellulase-producing bacteria from termites, caterpillars, and silk were isolated and screened. By interpreting the zones around the powerful colonies, cellulose production was qualitatively examined. It was also quantitatively estimated using the DNSA approach. A total of 3 bacterial isolatesas well as 2 actinomycetes were revealed to have high cellulase activity. Additionally, these isolates were examined for their ability to produce bioethanol utilizing various fruit wastes as a substrate. While using sweet lime waste as a substrate, isolates were discovered to be strong ethanol producer. The ability of isolates to treat wastewater from the paper and pulp industries was also examined. For this, a number of parameters including COD, DO, TS, TDS, Water hardness was examined and isolates was found to be efficient for waste water treatment.
Key words: *Cellulase degrading bacteria, bioethanol, waste water treatment.*

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INTRODUTION

The most common natural polymer on Earth is cellulose which is used as a structural element and is frequently coupled to other polymers [1-2]. The main component of most terrestrial plants' cell walls is cellulose[2]. An enzyme known as cellulases commonly decomposes cellulose. Numerous bacteria, both cell-bound and extracellular, produce the inducible enzymes cellulases when they develop on cellulose materials[3]. A variety of microbes, most frequently bacteria and fungus, synthesize this enzyme[2]. Determining appropriate methods for effective treatment using wastes containing cellulose as cheap carbon sources has therefore been of great economic significance [2,4]. Because of the variety of uses for them, cellulases have generated a lot of interest [3]. Cellulase is used in the manufacture of animal feed, the creation of detergents, the clarifying of juice, the paper industry, and the making of wine. Cellulase is having approximately 8% of the demand for industrial enzymes globally, and that demand is anticipated to rise by 100% in the coming years. However, a number of parameters, including incubation temperature, incubation time , pH value, carbon and nitrogen supplies, were discovered to have an impact on the synthesis of cellulase(s) by various microorganisms [5].Large amounts of agricultural, industrial, and municipal cellulosic wastes have been building up or being used inefficiently as a result of the high cost of their utilisation procedures. The fundamental structural component of plant cell walls and the most prevalent carbohydrate in nature is cellulose, a polymer of glucose residues linked together by beta 1,4 links. Therefore, it has become of great economic importance to create methods for the efficient treatment and use of cellulosic wastes as cheap carbon sources. The enzyme known as cellulase is responsible for breaking down the beta 1,4 glycosidic bonds in the polymer to release glucose molecules. Agriculture-related wastes may comprise cellulose [2]. A problem with environmentally acceptable disposal has been brought on by the global expansion of the papermill industry, which has generated a lot of primary and secondary sludge. Paper mills are thought to produce between 300 and 350 million tonnes of sludge annually, ensuring its availability as the raw material for biofuel generation. If these paper sludges aren't correctly handled, they pose a serious threat to the environment as well as to aquatic and agricultural sectors [6].

MATERIAL & METHODS

Sample collection: Sample was collected from various sources including different insects as well as compost. The Insects included caterpillar, termites. Several termites from damp wood were collected from wood part & caterpillars from marigold plant. Another sample was collected from cow fresh dung.

Enrichment of Pure Culture:
The collected termites were used in the dissection preparation. Prior to dissection each termite was exposed to a germicidal UV lamp for 15 minutes with agitation of the open glass dish to ensure complete exposure of the dorsal and ventral areas.
Enrichment Culture media:
Cellulose Broth & cellulose agar was used for enrichment & isolation of microorganism. Streak plate method was used for obtaining pure microbial culture. For observation of zone of clearance of cellulase activity, 0.02% congo red was added in cellulose agar media. Enriched samples were inoculated on agar media and incubated at 37^0c [9].
Isolation:
After dissection, digestive tracts was chopped with a scalpel. Collected Caterpillar and silk worm were dissected by separating gut. These gut was washed with H_2O_2 , suspended in sterile saline then crushed with the help of sterile glass rod. Prepared suspension was inoculated into sterile cellulose broth and incubated for 96hrs on rotary shaker at room temperature. Compost sample was diluted in sterile saline and then inoculated into sterile cellulose broth and incubated for 96hrs at 45^0c on BOD incubator shaker [9]. The isolates were obtained by inoculating enriched sample on Cellulose agar. Among obtained isolates promising 3 bacteria namely B1, B2 and B3 and 2 actinomycetes A1, A2 were selected for further study. Identification of actinomycetes was carried out by slide culture technique
Cellulase production:
Enriched broth of CDO was centrifuged and crude enzyme was obtained as a supernatant.
Culture Conditions Optimization for activity of cellulase enzyme
Various parameters like effect of pH, Temperature, Incubation period, Substrate concentration, Carbon and Nitrogen source on cellulase production was studied [10].Production, extraction and estimation of cellulase i.e., Qualitative assay was performed by measuring zone of clearance and Quantitative assay was performed by determining the amount of reducing sugars liberated by using Dinitrosalisilic Acid (DNSA) method [9].
Application of Cellulose degrading organisms(CDO)
Cellulose degrading organisms were tested for their ability to produce Bio-ethanol from fruit waste and agricultural waste and also for their efficiency in waste water treatment of paper and pulp industry
Bioethanol production from fruit waste & agricultural waste: Peels of various fruits were taken as a substrate instead of readymade cellulose powder[8]. These fruits waste was chopped into small pieces and used as substrate for CDO instead of cellulose. Reducing sugar present in prepared media was estimated by DNSA method. Cultured broth was distillated by using distillation assembly and O.D was taken at 540 nm [7].
Waste water treatment of Paper and pulp industry
The ability of isolates to treat wastewater of paper and pulp industry was examined. For this, a number of parameters including COD, DO, TS, TDS, Water hardness was examined.

RESULT AND DISCUSSION
A total of 3 bacterial isolates namely B1, B2, and B3, as well as 2 actinomycetes, A1 and A2, were revealed to have high cellulase activity.

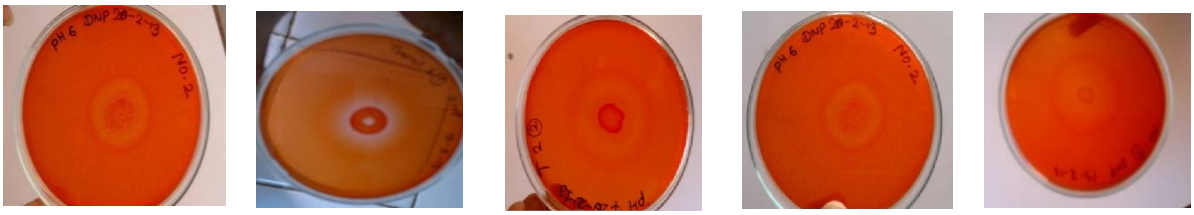

Figure 1: Zone of Clearance on Cellulose Agar Plate

Optimization of Culture Conditions
Effect of Temperature
Temperature effect on the cellulase enzyme activity of isolates were studied and it was found that all the isolates show high enzyme production at 37˚C temperature and isolate A2 was able to produce maximum cellulase followed by A1, B1, B3 and B2.

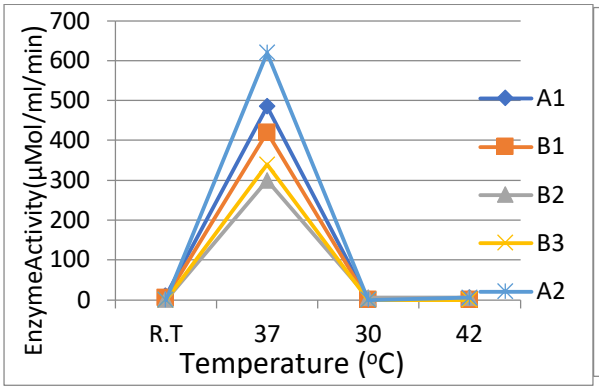

Figure2.Temperature effect

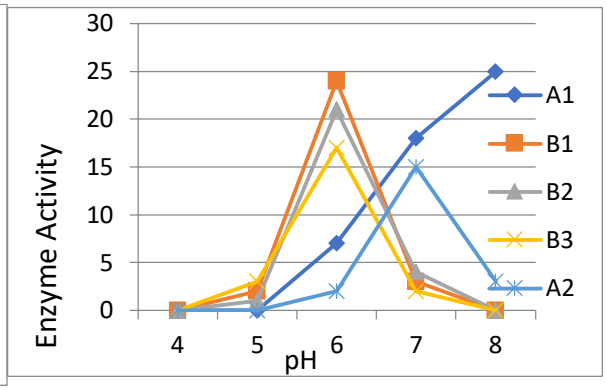

Figure3. pH effect

Effect of pH

At acidic pH no organism was able to show enzyme activity whereas most of the isolate i.e.B1, B2 and B3 shows maximum activity at pH 6. Isolate A2 shows maximum activity at Neutral pH. In case of A1 enzyme activity was increased as increase in pH and maximum activity seen at pH 8.

Incubation period

As incubation period was increased isolate A1 showed maximum enzyme activity while isolate B1, B2, B3 showed maximum activity by sixth day and it started declining as the period of incubation was increased.

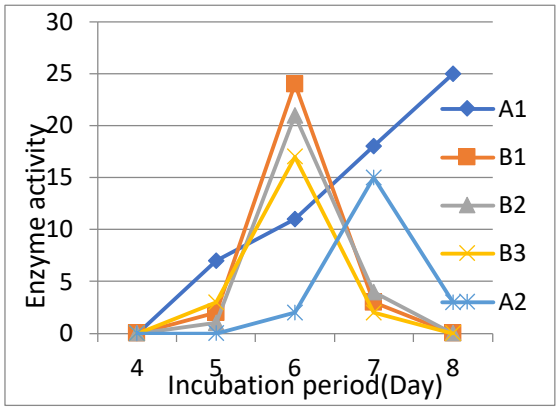

Figure4. Effect of Incubation period

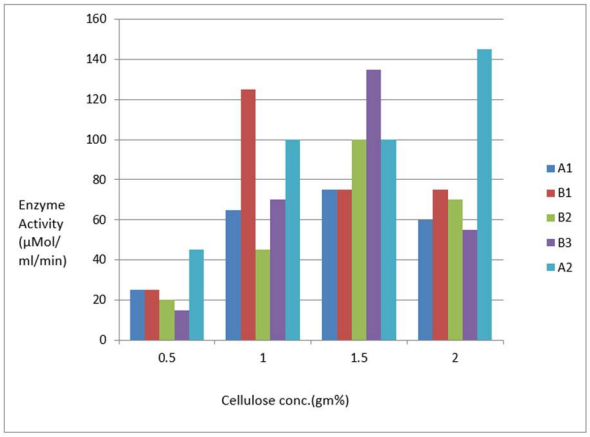

Figure5. Enzymatic activity

As the substrate conc. was increased isolate A2 showed maximum enzymatic activity.

Production, extraction and estimation of Cellulase enzyme

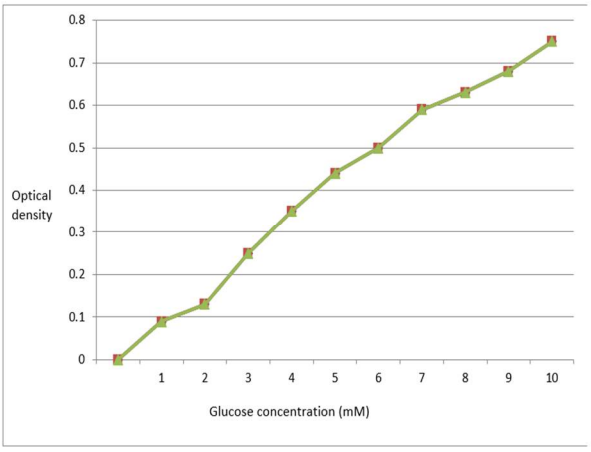

Figure6.a: Standard graph of glucose

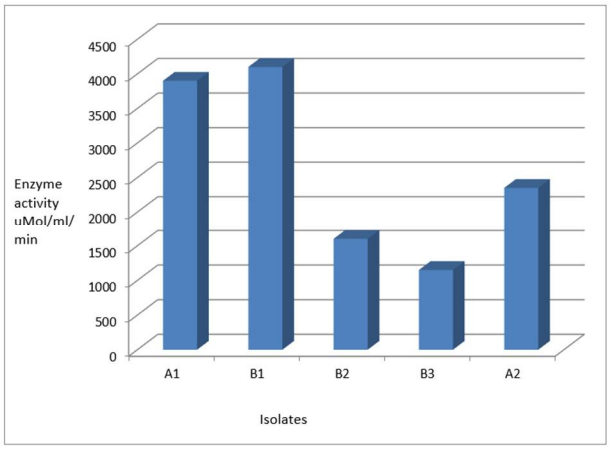

Figure6. b:Enzyme activity

Enzyme activity :-

Under the present study, enzyme activity of test organisms evaluated, it was found that, isolate B1 was potent for the cellulase production followed by isolate A1. While isolate B3 shows less enzyme activity as compared to other isolates.

Bio-ethanol production using CDO from Fruit Waste containing cellulose

All the isolates were tested for their ability to form bioethanol from different type of fruit wastes and it was found that all the isolates were capable of producing bioethanol when fruit waste is used as substrate. The amount of ethanol produced by organisms was different for each of these isolates and gram % of ethanol was found to be changed as change in substrate (Fruit waste). The comparative representation of gram % of ethanol produced by these 5 isolates using different type of substrates are mentioned below (Table 1)

Table 1: Comparative representation of ethanol production by Isolates grown on different fruit waste

Substrate	Gram % of ethanol				
	A1	B1	B2	B3	A2
Cellulose powder	6.3	4.2	3.5	8.4	4.9
Banana stem	7.7	11.2	11.2	11.9	7
Banana peel	10.5	7	9.8	13.3	7.7
Wheat straw	5.6	9.8	5.6	11.2	5.6
Water Melon	11.9	5.6	7	11.9	10.5
Mast Melon	11.9	11.9	8.4	11.9	12.6
Orange	14.7	9.8	17.5	14.7	14
Sweet lime	17.5	18.2	21.7	21	18.9

Current study shows that, all isolates were efficiently produces ethanol when grown on sweet lime waste and highest production of ethanol (21.7%) was observed by isolate B2. When organisms grown on Cellulose powder lowest ethanol production was observed as compared to other fruit wastes.(Graph)

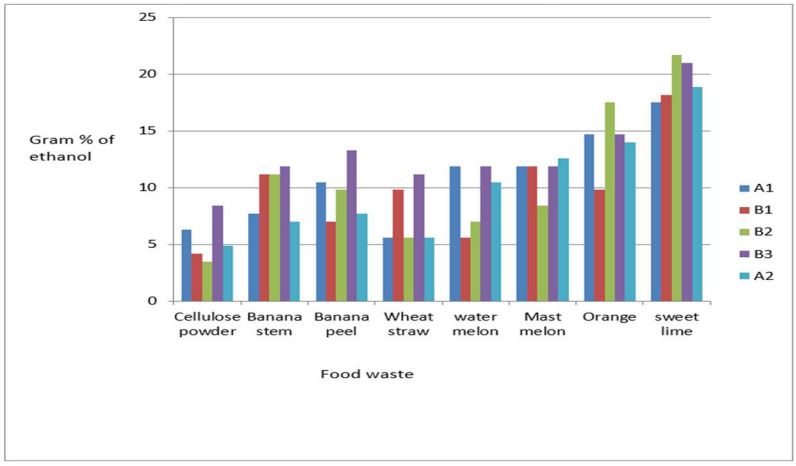

Figure 7: Comparative representation of ethanol production by Isolates grown on different fruit waste

Application of CDO in waste water treatment

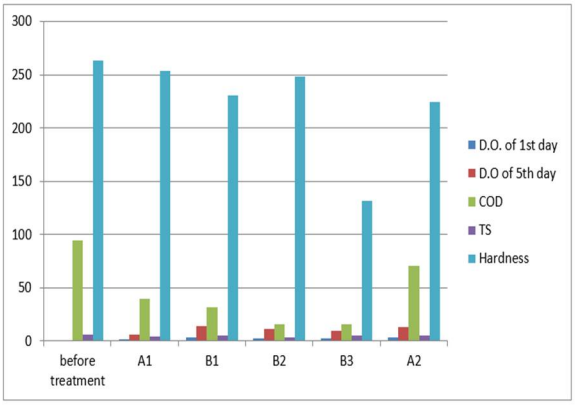

Figure 8: Effect on wastewater

In present study ability of isolates in waste water treatment was examined, It was found that B2 isolate shows maximum potency in wastewater treatment as compared to other isolates.

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