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Optimization for Cellulase Production from Soil by Using *Cellulomonas*

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Abstract

Enzymes are biological catalysts. Cellulase are used to hydrolyze cellulose and most abundant bio-polymer and potential source of utilizable sugars which serve as a raw material in the microbial population for wide variety of chemicals, foods and fuel. These enzymes are an important role in various industries like detergent, textiles, papers, biofuel production, degradation of wastes and fermentation industry. In this study the bacteria *Cellulomonas* produces cellulase enzyme in solid state fermentation at room temperature for 1-2 days which was isolated from soil and maintained on nutrient agar medium. Various agricultural wastes were used in shaker flask such as sugarcane, saw dust, coir, rice bran and paddy straw. They have sun dried, crushed and sieved to get the powder form which was pretreated with alkali. The sugarcane bagasse showed more enzyme activity (113.3µg/ml). Then enzyme characterization was studied by different parameters such as pH (6), temperature (45°C), carbon source (lactose), Nitrogen source (ammonium chloride), substrate (sugarcane bagasse) and incubation time. Enzyme activity was confirmed by Lowry's method and presence of reducing sugar followed by TLC. From those results, *Cellulomonas* is used for cellulase production to the industrial purpose which has novel application in production and processing.

Keywords

Cellulase, *Cellulomonas*, Pretreated, Solid state, Sugarcane

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INTRODUCTION:

Cellulose is most abundant and renewable biopolymer on the earth which is degraded by cellulase enzyme. It has a complex-multi enzyme system and they are mainly 3 types namely Endo β-Glucanase, Exo-β Glucanase and β-Glucosidase which act directly on cellulose, originating mainly cellobiose and glucose which is then hydrolyzed into glucose by β-glucosidase [5] Cellulase are used in many industries. In recent years many advances have been made in developing acid hydrolysis process for cellulose hydrolysis [2, 8]. Cellulose is an insoluble, high molecular weight linear

polymer of D-glucose residues linked by β-1,4glucosidic bonds. It is the most abundant naturally polymerization usually varying between 3500 and 10,000 [13]. Generally, bacteria are known to produce only carboxyl methyl cellulose & only a few exceptions produce β-glucosidase & some strains of *Cellulomonas* and *Micrococcus* are known to synthesize cellulolytic, xylanolytic & β-glucosidic enzymes [16].

The bioconversion of various complex cellulosic waste materials such as bagasse [7] saw dust [14] coir pith, paddy straw, rice bran and various others have been reported. Biodegradability of these substrates can be enhanced by pretreatment like acid (Grethelin, 1985) or alkali treatment (Jackson, 1977; Van Soast, 1994) ammonia and urea Physical grinding and milling (Ladisch et al., 1983; Fashey et al., 1992). Cellulose expression is subject to catabolite expression by glucose at concentrations higher than approximately o.1g/L [14] Solid substrate fermentation (SSF) deals with utilization of water insoluble materials for microbial growth and metabolisms. In simple cultivation equipment, with improved product recovery, water output, and at lower capital 7 operating expenses [4]. Cellulase production and activities are based on several factors such as carbon, nitrogen & phosphorous sources, the ratio of carbon to nitrogen provided, trace elements pH and aeration rate [1, 11and 19]. Chemical pretreatments include exposure of cellulose to alkali (NaOH) and dilute acid hydrolysis. The use of dilute acid (0.8 2% w/w H_2SO_4) has proved to be efficient (Groh Mann *et al*., 1985). Lactose has been reported to enhance cellulose productivity and yield when compared with cellulose (Freein, 1986)

The presence of Tween-80 reportedly results in enhanced cellulose production by increasing the permeability of the cell membrane and this increasing enzyme secretion [19]. It was found that a well-defined media containing lactose, ammonium salts and minerals [18] supported the growth of organisms. Rich undefined media of fructose or glucose of organism as carbon source [14] and yeast extract (Meyer and Humphrey, 1982) as nitrogen sources seem to stimulate cell growth and cellulose synthesis.

Alkali treatment was investigated by Jackson in 1977 (Millet *et al*.,1976, Toyama, 1970, Ghedaliya,1981) studied that pretreatment of cellulosic biomass to achieve effective scarification by cellulase. Ammonia and urea treatment was studied by (Busaglia *et al*,.1992, Van Soest,1994). Cellulolytic bacteria was shown to degrade cellulose at rates comparable to thermophilic bacteria [9, 20] the protein was determined by Lowry et al.,1951. From the presented view it is evident that we first need to understand the kinetics of cell growth and enzyme synthesis, using the most promising currently available microorganisms.

MATERIALS AND METHODS:

Source of Microorganism

The present study *Cellulomonas* sp was isolated from soil sample from Namakkal, Tamilnadu. For the production of cellulase enzyme which was identified by biochemical characterization and morphological examination. The bacterial culture is maintained on nutrient agar medium

Collection of substrates

Different agricultural wastes such as coir pith, sugarcane bagasse, saw dust, rice bran, paddy straw were collected and used as substrate by solid state fermentation. (pretreatment substrate and raw substrate).

Pretreatment of the substrates

The raw substrates were sun dried individually to reduce the moisture content to make the more susceptible for crushing. The crushed substrates are then sieved to get the powder form. Then the substrates were soaked individually in 1% sodium hydroxide solution (NaOH) in the ratio 1:10 (substrate solution) for 2 hours at room temperature after which they were washed free of chemicals and autoclaved at 121°C for an hour. The treated substrates were then filtered and washed with distilled water until the wash water becomes neutral.

Screening of the organism - Congo red assay

The CMC (Carboxyl Methyl Cellulose) plate was prepared which contained the isolated colonies were flooded with Congo red solution. They were incubated at room temperature for half an hour. The Congo red solution was drained off. The excess Congo red solution was washed by 1M NaCL. The colony was observed for cleared zone around. Hence the cellulolytic activity was conformed.

Preparation of Inoculum

100 ml of optimized culture media is prepared, 10 g of the substrates was dispensed. Separate media were prepared for each of the substrates and both

pretreated as well as raw substrates are used for inoculation. The various media containing the different solid substrates were sterilized in an autoclave at 121°c for 15 minutes at 15lbs. The solid substrates were inoculated with the *Cellulomonas* organism individually. The inoculum of bacterium was obtained from cultures growing on nutrient agar medium. Single colony of *Cellulomonas* is prepared which was utilized.

Method of inoculation

To culture suspension (0.5ml) was sprayed over the sterile solid substrate and mixed thoroughly by shaking on different substrates by shaking and incubated at room temperature for 1-2 days then they were utilized for enzyme extraction.

Enzyme Extraction

The inoculated media was filtered by cheese cloth to remove solid particles. The filtrate collected and centrifuged to obtain the enzyme extract. The enzyme extract was stored at 4°C till use [15]. The enzyme protein was quantified by Lowry's method.

Enzyme activity in CMC plate

The plate activity was determined with 1% CMC plate. The plate containing 1.5g agar in phosphate buffer and ammonium nitrate was used. The enzyme was dropped into the well and kept for incubation stained with Congo red and destained with 1% NaCL.

OPTIMIZATION OF CULTURE CONDITIONS FOR ENZYME PRODUCTION:

Time course for Enzyme production

The enzyme production at different intervals was determined. The enzyme productivity by *Cellulomonas* sp was checked on 1st, 2nd, 3rd, 4th, 5th, 7th, 9th, and11th day of incubation.

Effect of pH on the production of cellulase

The optimized media was prepared using the individual substrates pH was set at different levels from acidic to basic by adding 1% NaOH & concentrated Hcl. It was sterilized later they were inoculated with the *cellulomonas* organisms & kept for incubation. For *Cellulomonas sp* the pH of the media was set at 4, 5, 6 and 7. The enzyme production was determined by Lowry's method.

Effect of temperature on cellulase production

The optimized media was prepared individually by using substrate and sterilized. Later it was inoculated with the organisms separately and was incubated at different temperatures such as 4˚C, 37˚C, 45˚C, and 60˚C. Then the enzyme activity was checked.

Effect of carbon source on cellulase production

The effect of different carbon sources such as glucose, sucrose, lactose, and starch were used individually by adding 0.1gm of each of them into the optimized media. The media was inoculated and then kept for incubation.

Effect of Nitrogen source on cellulase production

The effect of different nitrogen sources were studied by adding 0.1gm of ammonium sulphate, sodium nitrate, urea and ammonium chloride individually to the optimized media containing the different solid substrates. The media was inoculated with the organisms and then kept for incubation. Then the enzyme activity was determined.

RESULT:

Screening of the organisms – Congo Red Assay

The cellulolytic activity was confirmed on carboxy methyl cellulose plate which has produced cleared zones around the colonies. (Fig.1and 2).

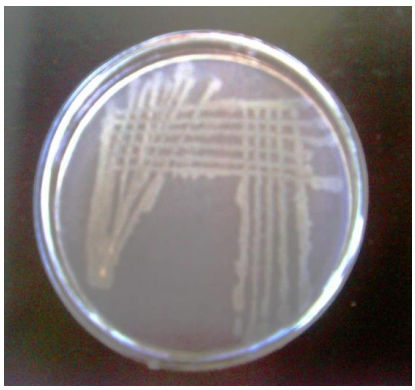

Fig.1 Cellulomonas Bacteria

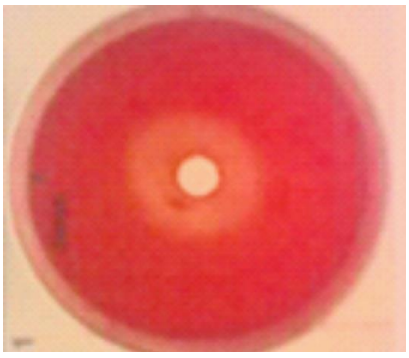

Fig.2 CMC Plate shows Zone of activity

Different types of agricultural wastes were used such as sugarcane bagasse, coir pith, saw dust, rice bran and paddy straw. Both pretreated as well as raw substrates were used. The preparation of solid-state

fermentation system, inoculation, extraction & assay of enzyme were mentioned in the materials & methods cellulolytic activity was determined by Lowry's method. (Fig.3).

Enzyme production on different solid substrates:
The following optimum parameters were shown such as pretreated substrates, raw substrates, p^H, Temperature, Carbon & Nitrogen Source (Fig 1-6)

Fig.3 After Incubation Period (1-2days)

Fig.1 PRETREATED SUBSTRATES

S. No	Pretreated Substrates	Cellulase activity (U/ml)
1	Sugarcane bagasse	133.3
2	Saw dust	**20**
3	Coir	**20**
4	Rice bran	**19.4**
5	Straw	**53.3**

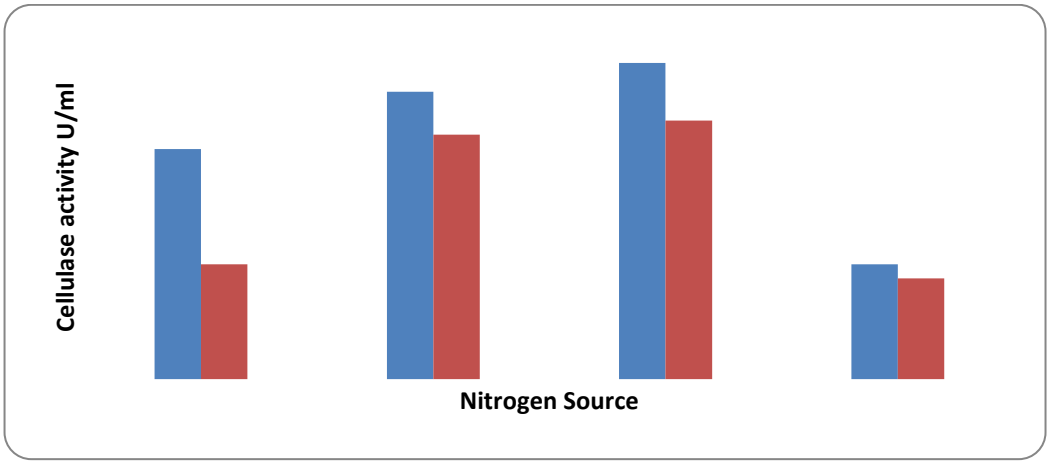

Fig 1: Effect of Pretreated Substrates

Fig.2 RAW SUBSTRATES

S.No	Raw Substrates	Cellulase activity(U/ml)
1	Sugarcane bagasse	101.2
2	Saw dust	**14.8**
3	Coir	**15.4**
4	Rice bran	**11.5**
5	Straw	**42**

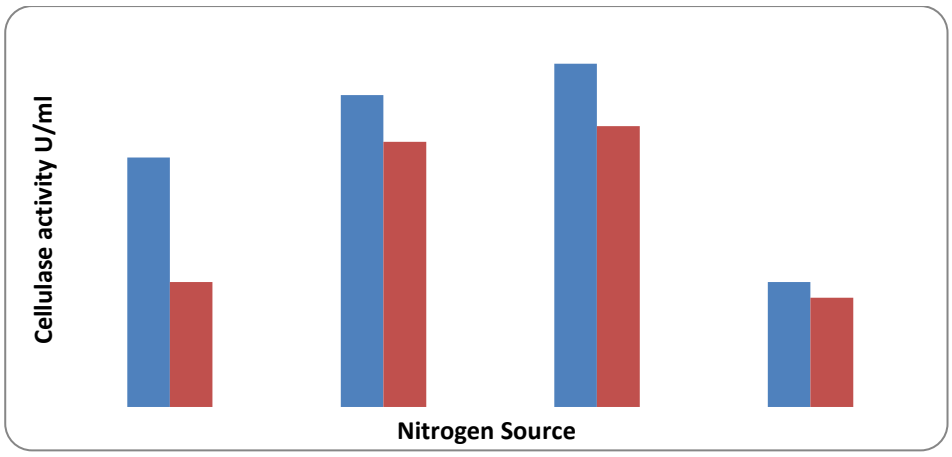

Fig 2: Effect of Raw substrates

Fig.3 pH

S.No	pH	Sugarcane bagasse (U/ml)	Paddy straw (U/ml)
1	4	**53.33**	**53.33**
2	5	**113.33**	**80**
3	6	**133.33**	**120**
4	7	**73.33**	**80**

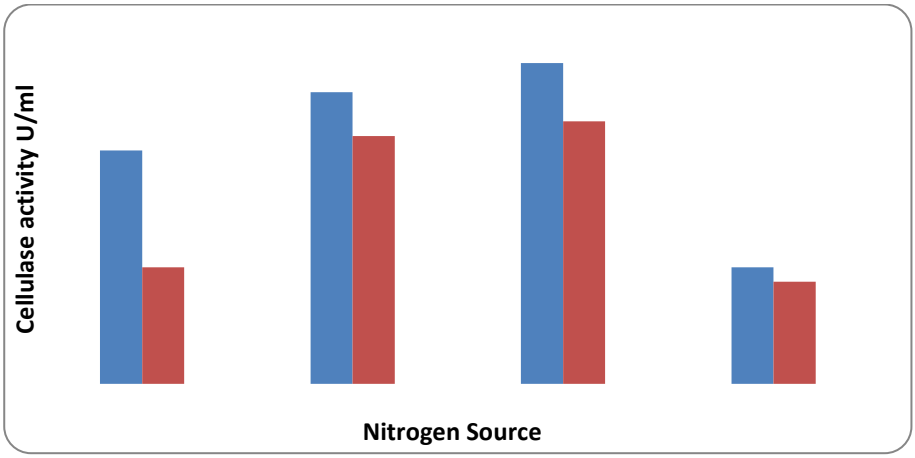

Fig 3: Effect of pH

Fig.4 TEMPERATURE

S.No	Temperature	Sugarcane bagasse	Paddy straw (U/ml)
1	4	**53.33**	**46.66**
2	37	**106.66**	**106.66**
3	45	**120**	**113.33**
4	60	**26.66**	**20**

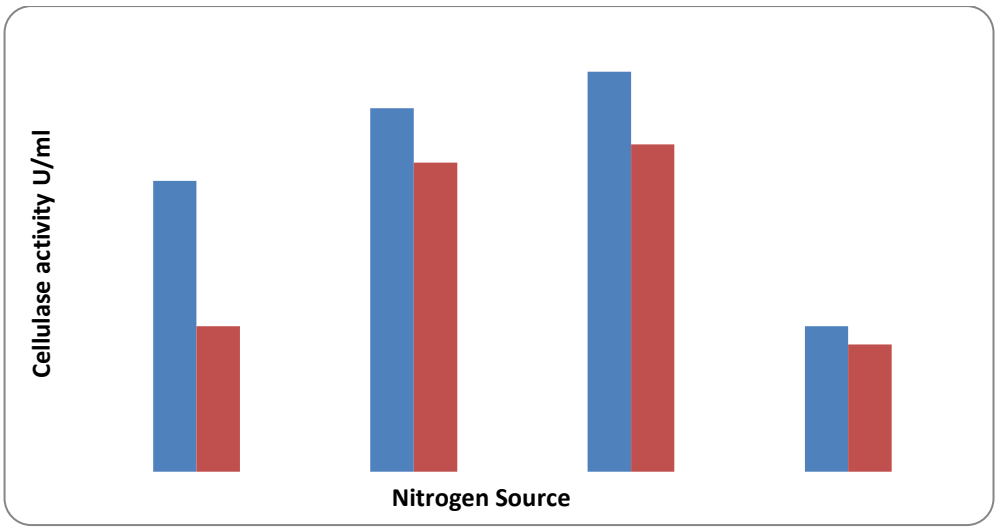

Fig 4: Effect of Temperature

Fig.5 CARBON SOURCE

S.No	Carbon source	Sugarcane bagasse (U/ml)	Paddy straw (U/ml)
1	Glucose	**133.33**	**120**
2	Lactose	**153.33**	**166.66**
3	Sucrose	**106.66**	**80**
4	Starch	**113.33**	**73.33**

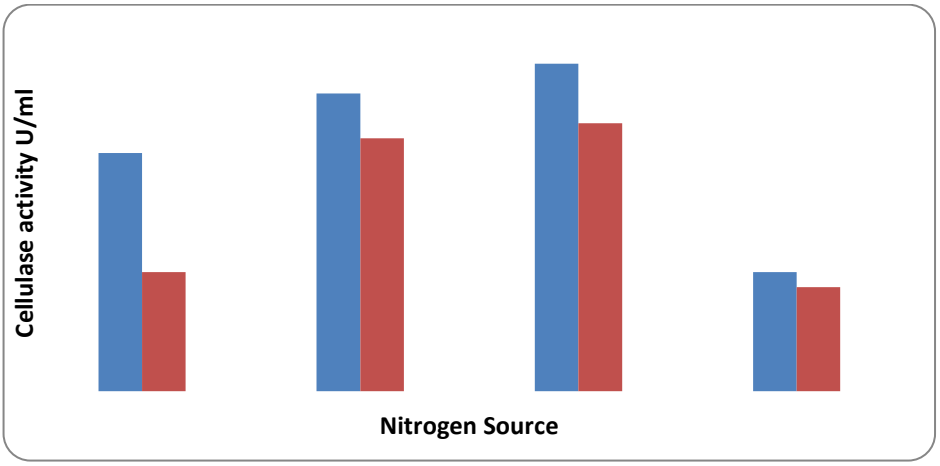

Fig 5: Effect of Carbon source

Fig.6 NITROGEN SOURCE

S.No	Nitrogen source	Sugarcane bagasse (U/ml)	Paddy straw (U/ml)
1	Urea	**106.66**	**53.33**
2	Ammonium sulphate	**133.33**	**113.33**
3	Ammonium chloride	**146.66**	**120**
4	Sodium nitrate	**53.33**	**46.66**

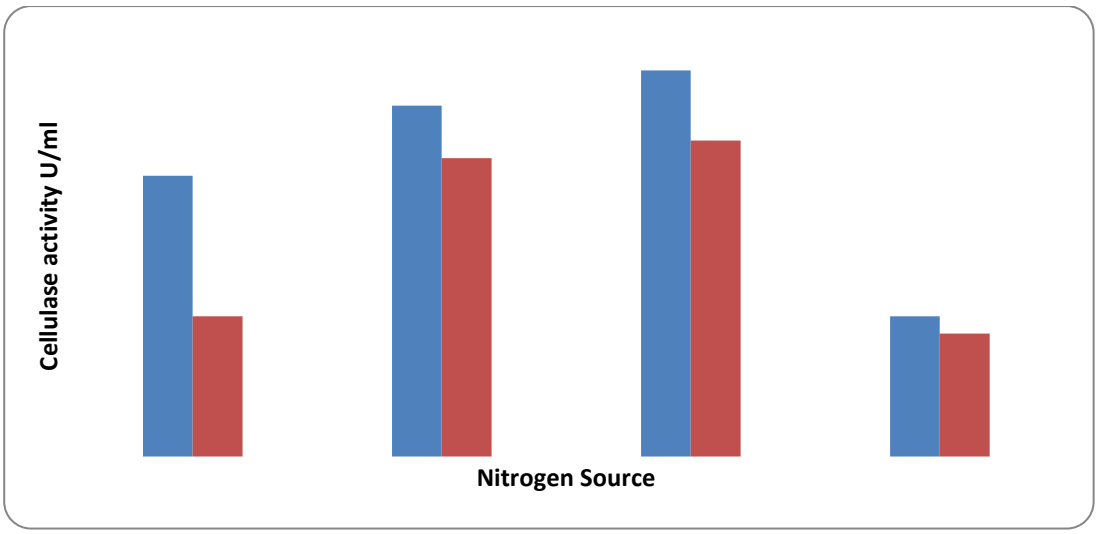

Fig 6: Effect of Nitrogen source

DISCUSSIONS:

The research should also aim at exploiting the commercial potential and new cellulose in nature [3]. Agricultural residues such as rice bran, paddy straw, sugarcane bagasse, coir pith and saw dust were use in cellulase production [12] although the raw materials are cheaper, pre-treatment generally required to improve the utility of lingocelllosic materials, and the cost is considerable [10] the raw materials were utilized. Some environmental factors also increase the growth of organisms as well as maximum production of enzyme will be at certain optimum temperature, pH [6]. The presence of reducing sugar is confirmed by thin layer chromatography using silica as an adsorbent material.

CONCLUSION:

The *Cellulomonas* sp is isolated from soil sample and their cellulolytic activity was confirmed on CMC plate which has produced cleared zones around. Different types of agricultural residues were used for cellulase enzyme production. Pretreatment substrate has showed better result. In that pretreatment substrate sugarcane bagasse has showed more enzyme activity. Presence of reducing sugar is confirmed by TLC and protein is quantified by Lowry's method and the supernatant was used as enzyme source and different parameters were studied optimum pH for *Cellulomonas* is found to be 6, the best substrate sugarcane bagasses found to be more effective in cellulase production and maximum incubation time for production of *Cellulomonas* 1 to 2 days. The optimum temperature is 45^{o}c for *Cellulomonas* for enzyme production. The nitrogen source (Ammonium chloride) and carbon source (lactose) were used for best cellulase enzyme production. The basic idea was to enhance the cellulase production from microorganisms with low cost.

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