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Isolation, characterization of cellulolytic bacteria and its application in waste treatment

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Cellulolysis is the process of hydrolysis of cellulosic material with the help of cellulose degrading enzyme i.e. cellulase. The microbial method employed for cellulose dergradation offers potentially efficient and affordable technique. The goal of this research work was to exploit the cellulolytic potential (cellulases) of bacterial isolates for the treatment of textile effluent.

Key words : Textile waste, Colour, Cellulases, Industrial effluent, Biosorption

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INTRODUCTION

Cellulases are inducible enzymes which are synthesized by microorganisms during their growth on cellulosic materials (Lee *et al.,* 1996). Cellulase hydrolyzes the β-1, 4-glycosidic bonds in the polymer to release glucose units (Nishida *et al.,* 2007).This cellulose degrading enzyme can be used in the formulation of washing powder, extraction of fruit and vegetable juices and starch processing. Cellulases interactively promote the cellulose degradation (Wood and McCrae, 1982; Mishra and Rao, 1988), to cope with the problems of food and energy shortages expected in near future with explosive increase in human population.

Isolation of cellulolytic bacteria:

Cellulose producing bacteria were isolated from wood samples on the basis of their ability to grow on cellulose containing media *i.e.* CMC. Isolation of cellulase producing bacteria was done using CMC agar medium. Samples were incubated at 37^0 C for 24 hours. The plates were stained with Congo red solution and destained with 1M NaCl solution. Clear zone indicated the hydrolysis of CMC as a result of cellulases production. Then pure bacterial colonies were cultured in flasks containing LB-CMC broth.

Characterization of isolated bacterial strain:

The identification and characterization of the isolated cellulolytic bacterial organisms were carried out according to the methods of Cullimoore, (2000) and Cowan and Steel, (1993). Isolated bacteria were gram negative, rod-shaped and formed glistening colonies on CMC agar plates. The strains showed maximum growth after 24 hour of incubation (with highest optical density *i.e.* 0.292). This shows that at 24th hour of incubation, there occurred maximum growth rate and product formation.

Biochemical tests of isolated bacterial strain:

Biochemical characterization of the isolates revealed it to be positive for urease, indole production, methyl-red and glucose and sucrose fermentation. The isolates showed negative result for the citrate utilization, catalase activity, voges-proskauer and lactose fermentation (Table 1).

Table 1: Biochemical tests of isolated cellulolytic bacteria

Sr. No.	Characters	Observation	Results
1.	Urease test	Yellow to red/pink	+ve
2.	Indole production	Formation of red layer	+ve
3.	Methyl-red test	Remains red	+ve
4.	Voges-Proskauer test	Red to yellow	-ve
5.	Citrate utilization	Remains green	-ve
6.	Catalase activity	No bubbles released	-ve
7.	Carbohydrate fermentation		
	Glucose	Red to yellow	+ve
	Lactose	Remains red	-ve
	Sucrose	Red to yellow	+ve

Enzyme assay (DNSA Method) :

Enzyme activity was determined by DNSA method. A standard glucose curve (Fig. 1) of optimal density against glucose concentrations was plotted to obtain a standard calibration curve from which the sugar concentrations of unknown samples (test samples) were extrapolated using their respective absorbance by varying parameters like pH, temperature, incubation time and substrate concentration (Otajevwo and Aluyi, 2011).

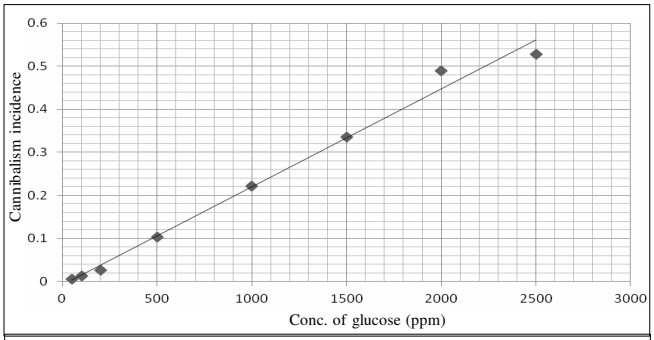

Fig. 1: A standard glucose curve

Effect of pH and temperature on enzyme activity of isolated cellulolytic bacteria :

Microorganism grows at optimum pH which is suitable for substrate consumption as well as product formation. To observe the effect of pH on cellulose activity the fermentation was carried at various pH levels (Table 2).

Table 2: Effect of varying pH on enzyme activity of isolated cellulolytic bacteria

Sr. No.	pH	E.A. (U/ml)
1.	4	0.071
2.	5	0.142
3.	6	0.230
4.	7	0.195
5.	8	0.100

Optimal pH of isolated cellulolytic bacteria is comparable to cellulase of *Paenibacillus* sp. which has maximum CMC activity at pH 6.5. Due to the slight acidic tolerance of this enzyme, it has good potential for industrial use in the hydrolysis of soluble cellulose as well as activity on microcrystalline sources of cellulose (Wang *et al.,* 2008).

The enzyme showed a good activity between 30 to 40^{0}C with maximum activity at 35^{0}C, which confirms that it is a mesophilic bacterium (Table 3). This is comparable to *Bacillus circulans* having optimal cellulolytic activity at 35^{0}C whereas *Clostridium thermocellum* shows optimum enzyme activity at 50^{0}C (Otajevwo and Aluyi, 2011).

Table 3: Effect of temperature on enzyme activity of isolated cellulolytic bacteria

Sr. No.	Temperature (^{o}C)	E.A. (U/ml)
1.	25	0.083
2.	30	0.122
3.	35	0.241
4.	40	0.180
5.	45	0.107

Effect of substrate concentration on enzyme activity of isolated cellulolytic bacteria :

Initially as substrate concentration increases, the speed of the reaction also increases. This happens because free activation centre of the enzyme binds to free substrates. Once all activation centres of the available enzymes bound to their substrates, new increments of the substrate concentration will have no effect on the speed of the reaction.

Bacteria showed maximum enzyme activity, incubated with CMC having concentration 1 per cent (Table 4).

Table 4: Effect of substrate concentration on enzyme activity

Sr. No.	Substrate concentration (%)	E.A. (U/ml)
1.	0.5	0.18
2.	1.0	0.26
3.	1.5	0.21
4.	2.0	0.15

Application of isolated cellulolytic bacteria in textile waste treatment :

To check the potential activity of isolated bacterial culture, it was used in the treatment of textile waste water treatment. Textile effluent was procured to perform various parameters analysis like colour greenish, pungent smell, pH 7.8, COD 740mg/l, TS 3700mg/l, TDS 2300mg/l and TSS 1400 mg/l according to standard methods (APHA, 1995).

Effect of concentrations of textile waste water on growth of isolated cellulolytic bacteria :

The purpose of adaptation is to allow the bacterial culture to rapidly adapt to their new environment. Adaptation studies were carried out by growing the bacteria in CMC broth with 1 per cent, 2.5 per cent, 5 per cent, 7.5 per cent and 10 per cent of textile wastewater.

Table 5: Effect of concentration of textile wastewater on growth of isolated strains

Sr. No.	Textile wastewater (%)	Absorbance (O.D. at 600nm)
1.	1.0	0.16
2.	2.5	0.24
3.	5.0	0.11
4.	7.5	0.05

From 1 per cent to 2.5 per cent textile wastewater, the OD_{600nm} increased (Table 5). Upon further increasing the textile wastewater concentration up to 7.5 per cent, there was observed a decrease in the OD value. High OD value indicated the cells are increased in populations (Madigan *et al.,* 2000).

Effect of % inoculum of isolated bacterial strain on degradation of textile waste water:

There was maximum enzyme activity, when 7.5 per cent inoculums was used. So 7.5 per cent inoculum was optimum concentration for the cellulase production and degradation of textile effluent (Table 6).

Table 6: Effect of % inoculum on degradation of textile waste water

Sr. No.	Inoculums (%)	O.D. at 600nm
1.	2.5	0.237
2.	5.0	0.284
3.	7.5	0.358
4.	10.0	0.338

Effect of incubation time on COD removal:

Culture adapted to 2.5 per cent textile wastewater (7.5% inoculum) was introduced to the textile effluent without pH adjustment and incubated for 10 days.

Maximum COD reduction (43.1%) was found on 10th day of incubation (Table 7). When the textile effluent was inoculated with isolated cellulolytic bacteria, there was observed considerable decrease in the COD level. After every two days, COD was measured with the standard method. On 10th day of incubation, COD was found to be 421mg/l. Hence there occurred 43.1per cent COD removal by the isolated cellulolytic culture which is comparable with the biological technique proposed by Babu *et al*. (2000) who achieved 62 per cent of COD removal using mixed bacterial culture and 42.4 per cent COD reduction was reported by Kim *et al*. (2003) for cellulolytic bacteria (*Cellulomonas cellulans*).

Table 7: Effect of incubation time on % COD removal

Day	COD (mg/l)	% removal of COD
0	740	-
2	658	12.43
4	597	20.6
6	532.8	28
8	473.6	36
10	421	43.1

Conclusions:

The research work shows that the isolated cellulolytic bacteria have potential to resolve the problem of high COD Considerable reduction in all parameters was observed when the textile effluent was treated with isolated cellulolytic bacteria. The pH of the effluent sample after treatment was almost at neutral level *i.e*. 6.9. Total solids, total dissolved solids (TDS) and total suspended solids (TSS) in the textile effluent were reduced to 24 per cent, 21per cent and 28 per cent, respectively. The COD values were decreased by 43.1per cent in the effluent sample after treatment. Exploitation of isolated cellulolytic bacteria from decay wood sample for the degradation of textile effluent presented a cheap and efficient method. Such biological treatment method is cheap and offers the best alternative with proper analysis and environmental control.

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