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Isolation and Screening of Cellulolytic Bacteria Inhabiting Different Environment and Optimization of Cellulase Production

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Abstract:

The cellulase producing bacteria were isolated from various region including paper industry waste, municipal waste, sugarcane farm, garden, and wood furnishing region. Total 34 isolates were obtained by the primary screening technique from which 11 isolates were showing maximum cellulase activity. Potential isolates were obtained from wood furnishing region and paper industry waste. These 11 isolates were then evaluated by secondary screening for enzyme production. Among these 11 isolates CDB27 and CDB30 were selected as most efficient enzyme producers and their specific enzyme activity in the crude sample was found to be 6.0U/mg and 8.4 U/mg and of partially purified sample was found to be 6.97 U/mg and 9.3 U/mg respectively. Isolates were tentatively characterized on the basis of their cultural and morphological and biochemical characteristics, CDB27 and CDB30 were identified to be *Pseudomonas sp*and *Bacillus sp* respectively. Further partial purification of the cellulase enzyme was carried out by ammonium sulfate precipitation followed by dialysis. Optimization of different parameters was carried out for the production of cellulase by both efficient isolates. The maximum enzyme producing isolate CDB30 was used to check biodegradation properties at laboratory scale.

Keywords:*Bacillus sp.,*Biodegradation,Cellulase, Optimization, Partial purification, *Pseudomonas sp,*.

1.0 Introduction:

The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest (Bhat ,2000).It is the primary product of photosynthesis in terrestrial environments, and the most abundant renewable Bioresource produced in the biosphere(100 billion dry tons/year) (Zhang and Lynd, 2004). Approximately 70% of plant biomass is locked up in 5- and 6-carbon sugars (D- xylose, D- arabinose, D- glucose, D- galactose, D- mannose) which are found in lignocellulosic biomass comprised of mainly cellulose, lesser hemicelluloses and least of all lignin (Sadhu and Maiti, 2013). It is possible to convert this biopolymer into monomeric molecule glucose by both chemical and biological means. Cellulose the largest component of plant residues enters terrestrial ecosystems (Richmond, 1991) and therefore represents a huge source of energy for microorganisms, the main agents responsible for soil organic matter decomposition (Lavelle and Spain, 2001). Microorganisms including bacteria, fungi and actinomycetes are able to carry out bioconversion of cellulose. Cellulose, a crystalline polymer of D-glucose residues connected with β-1, 4 glucosidic linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature (Saha*et al.,* 2006).Hence, it has become a considerable economic interest to develop processes for effective treatment and utilization of cellulosic wastes as inexpensive carbon sources (Shanmugapriya*et al*., 2012). Cellulase is an enzyme used for the bioconversion of cellulosic and lignocellulosic residues. Cellulolytic activity is a multi-complex enzyme system and complete enzymatic hydrolysis of enzyme requires synergistic action of 3 enzymes;endo-•-glucanase (EC 3.2.1.4), exo-•-glucanase (EC 3.2.1.91) and •–glucosidase (EC 3.2.1.21) (Shankar andIsaiarasu, 2011). These enzymes act sequentially in the synergistic system and subsequently convert cellulose into an utilizable energy source and glucose and hence cellulases provide a key role in biomass utilization. Mainly efficient cellulase activities are observed in fungi but there is increasing interest in cellulase production by bacteria because bacteria have high growth rate as compared to fungi and has good potential to be used in cellulase production. The

search for a novel and improved bacterial strain, having hyper cellulase productivity with more activity and high stability against temperature, pH and under non-aseptic conditions might make the process more economical.Bacteria and fungi have been found to produce and secrete these enzymes freely in solution; however, some microorganisms have also been found to produce cell-bound enzymes and multi-protein complexes expressing cellulases and hemicellulases called cellulosomes. The cellulosome was first discovered in 1983 from the anaerobic, thermophilic spore-forming *Clostridium thermocellum* (Maki *et al*., 2011). The production of cellulase generally depends on variety of growth parameters which includes inoculum size, pH value, temperature , presence of inducers, medium additives, aeration, growth and time (Immanuel *et al*., 2006) and also the cellulase activity is appear to be depend on the presence of various metal ions as activators and inhibitors (Muhammad *et al.,* 2012).Cellulases have numerous applications in the area of industry and pharmaceuticals. The major industrial applications of cellulases are in textile industry for 'bio-polishing' of fabrics and producing stonewashed look of denims, in household laundry detergents for improving fabric softness and brightness (Cavaco-Paulo, 1998), in food, leather, paper/pulp industries and also used in the fermentation of biomass for the boifuel production. Besides, cellulases are also used in ruminant nutrition for improving digestibility, in fruit juices processing and another emerging application is de-inking of paper (Sakthivel*et al.,*2010). These industrial applications focused on to the cellulases which can be highly stable and in active state at extreme pH and temperature. Certain cellulase producing bacteria are also inhabiting the Erthwormgut which are responsible for decomposition of organic matter and composting (Shankar *et al.,* 2011*)*.

Beyond free bacterial cellulases, is the opportunity for whole cells in bacterial co-culture and strains with multiple exploitable characteristics to reduce the time and cost of current bio-conversion processes. And also as the final product of cellulose degradation by cellulase enzyme is glucose wich is soluble sugar. So, isolation and characterization of cellulase producing bacteria will continue to be an important aspect of biofuel research, biodegradation and bioremediation.

The present study was attempted with the following objectives:

- To isolate and screen cellulolytic bacteria from different environmental sources.
- Production of cellulase from potential isolates by submerge fermentation process.
- Partial purification of cellulase and determination of its Enzyme activity and Specific activity.
- Optimization of different parameters for batter cultivation and production process.
- Application of potential isolate in biodegradation of cellulosic material.

2.0Method:

2.1Sample collection, isolation and primary screening for cellulase producing bacteria:

The soil samples were collected from the different areas such as garden soil, wood furnishing region and sugar cane farm and water samples were collected from paper industry waste and - municipal waste. The samples were collected in sterile container and stored at 4ºC until used.

Tenfold serial dilutions of each soil sample were prepared in sterilized distilled water and 0.1 ml of that diluted sample was spread on Carboxymethyl cellulose medium recommended by Ray *et al*. (2007). It has the following composition (g/l): Carboxymethylcellulose (CMC), 10; Tryptone, 2; KH_2PO_4, 4; Na_2HPO_4, 4; $MgSO_4.7H_2O$, 0.2; $CaCl_2$ $.2H_2O$, 0.001; $FeSO_4.7H_2O$, 0.004; Agar, 15 and pH adjusted to 7(Ariffin*et.al.,* 2006). All the plates were incubated at 37^0C for 3-5days. The plates were flooded with 1% Congo red and 1M NaCl to see the cellulolytic activity of isolated strain. The formation of a clear zone of hydrolysis indicated the cellulose degradation. The ratio of the clear zone diameter to colony diameter was measured in order to select for the highest cellulase producer (Ariffin*et.al.,* 2006). The largest ratio was assumed to contain the highest activity.

2.2 Maintananceof pure culture:

The colonies showing significant clear zone were plated on to the minimal agar medium and analyzed for colony characteristics and sub-cultured on to the minimal medium containing 1% CMC and incubated at 37˚for 24h and then stored at 4˚C (Immanuel *et al*., 2006).

2.3 Secondary screening and production of cellulase enzyme:

The potential isolates were then evaluated for enzyme productivity. Those isolates showing maximum cellulase production were then considered for the further study.

2.3.1 Submerge Fermentation process:

For preparation of standard inoculum, those isolates showed a maximum zone of hydrolysis were cultured in 20 ml inoculum medium[composition (g/l): Carboxymethylcellulose (CMC) 5; Tryptone 2; KH_2PO_4 4; Na_2HPO_4 4; $MgSO_4.7H_2O$0.2; $CaCl_2.2H_2O$ 0.001; $FeSO_4.7H_2O$ 0.004 and pH adjusted to 7] individually and incubated at 37 ºC for 24 h where an average viable count of 2-3.5x10^6 cells /ml culture was obtained. This was used as inoculum for the production medium.The composition of production medium was same as of inoculum medium except the concentration of Carboxymethyl cellulose which was 1% instead of 0.5%.

Fermentation was carried out in 250 ml Erlenmeyer flasks, each containing 100 ml sterile production medium and inoculated with 5% of standard inoculums (containing 2-3.5x10^6 cells /ml). The flasks were incubated at 37^{0}C on a rotary shaker at 150 RPM for 72h.

2.3.2 Preparation of crude enzyme:

After incubation, the cultures were centrifuged at 1600 RPM for 20 min at 4°C and supernatant was used as a source of crude enzyme. The crude enzyme solution was utilized for determination of enzyme activities.

2.4 Cellulase enzyme assay:

Carboxymethylcellulase(CMCase) activity was estimated using a 1 % solution of carboxymethlycellulose (CMC) in 0.05 M citrate buffer (pH 4.8) as substrate. The reaction mixture contained 1 ml citrate buffer, 0.5 ml of substrate solution and 1ml of crude enzyme solution. The reaction was carried out at 45°C for 30 min. The amount of reducing sugar released in the hydrolysis was measured by DNSA method.The Enzyme unit (EU) was determine as the amount of CMCase required to release 1µmole of reducing sugar per ml per minute under above assay condition.

2.5 Protein determination:

Protein concentrations in a crude sample were determined by using a Folin Lowry method (Lowry *et.al.,* 1951) with bovine serum albumin (BSA) as a standard.

2.6 Partial purification of cellulase enzyme:

2.6.1 Ammonium sulfate precipitation:

About 20 ml of the crude enzyme solution was saturated by solid ammonium sulfate and the mixture was left overnight at 4^{0}C for precipitation (Lee *et.al.,* 2008). The precipitates were collected by centrifugation and dissolved in 10 ml of 50 mM sodium acetate buffer (pH 5.5).

2.6.2 Dialysis:

For partial purification, enzyme collected after ammonium sulfate precipitation was dialyzed against 30mM sodium acetate buffer (pH-5.5) at 4ºC with three changes of buffer (Lee *et.al.,* 2008). The partially purified sample was assayed for enzyme activity and protein content.

2.7 Identification of cellulase producing bacteria:

Potential isolates were tentatively identified by means of morphological, cultural and biochemical characterization.

2.7.1 Morphological characterization:

For morphological characterization colonies were stained by Gram's staining technique and for suspected isolates special staining was also performed included capsule staining and endospore staining. Motility test was also performed.

2.7.2 Cultural characterization:

The pure culture of individual isolates were further characterized, on the basis of their Gram's reactivity individual isolate was passed on Nutrient agar and Mac Conkey's agar plate and then on special media. After incubation colony characteristics were noted.

2.7.3 Biochemical characterization:

Different biochemical tests were analyzed included Indole test, Methyl red test, Vogues-Proskauer test, Citrate utilization test, starch hydrolysis, gelatin liquefaction, nitrate reduction, Catalase test, Oxidase test, phenylalanine deamination and sugars fermentation test.

2.8 Optimization of cellulase production:

The optimum parameters were determined for cellulase production from the efficient isolates. The cellulase fermentation was carried out at different ranges of parameters included temperature, pH, incubation period, substrate concentration and inoculums size. After fermentation at different parameters the crude enzyme sample was collected from each to check the enzyme activity.

2.8.1 Effect of temperature:

To determine the optimum temperature for cellulase production, fermentation was carried out at various temperatures in the range of 25ºC, 35ºC, 45ºC, 55ºC and 65ºC.

2.8.2 Effect of pH:

Different values of pH ranged from 5 to 8 were chosen for studying their effects on cellulase enzyme production.

2.8.3 Incubation period:

To obtain maximum cellulase production fermentation was carried out at different incubation periods ranging from 48, 72, 96 and 120 hours.

2.8.4 Effect of substrate concentration:

To evaluate the effect of substrate concentration on cellulase production the production medium was supplemented with different concentration of CMC including, 0.2%, 0.5%, 1% and 1.5%.

2.8.5 Inoculums size:

The inoculum size was optimized for maximal enzyme production. The fermentation medium was inoculated with 1, 2, 4, and 6 % of standard young log phase inoculums(containing 2-3.5x10^6 cells /ml).

2.9 Application of most efficient cellulase producer in biodegradation:

Most efficient isolate was selected and used for filter paper and cotton degradation. For this a sterile mineral buffered solution with pH 7.5 was individually supplemented with filter paper strips and cotton as a sole source of carbon and the medium was supplemented with two drops of 10 mM glucose to possibly induce cellulase production(Maki *et al*., 2011). Then, the log phase culture of selected most efficient isolate was separately inoculated into this medium. The culture was incubated for maximum 6 days at 50^{0}C in shaking condition at 120rpm and observed daily for visual evidence of degradation.

3.0 Result and Discussion:

3.1 Isolation and primary screening for cellulase producing bacteria:

Total 15 samples were collected from 5 different sites and total 34 isolates were obtained as shown in Table-1. From these, 20 out of 34 isolates were removed due to similar colonial and morphological characteristics. The resulting 14 isolates were then tested on CMC agar for cellulase activity. Their CMCase activity is shown in Table-2.

Table-1 Different sites for sample collection for cellulase producers.

Site	Sample no.	Total no. of isolates	Labeled as..
Garden	S_1-S_3	12	CDB1,….., CDB12
Wood furnishing Region	S_4-S_6	6	CDB13,….., CDB18
Sugarcane farm	S_7-S_9	5	CDB19,…, CDB23
Paper industry Waste	S_{10}-S_{12}	7	CDB24, …,CDB30
Municipal waste	S_{13}-S_{14}	4	CDB31,…, CDB34

Table-2 Zone of hydrolysis of different isolates.

Sr no	Isolate no	Colony diameter (n) (mm)	Zone diameter (z) (mm)	(z/n) (mm)
1.	**CDB7**	**8.7**	**10.0**	**1.3**
2	**CDB11**	**7.5**	**9.0**	**1.5**
3.	**CDB14**	**5.6**	**7.0**	**1.4**
4.	**CDB16**	**10.6**	**12.0**	**1.3**
5.	**CDB17**	**8.0**	**10.0**	**2.0**
6.	CDB19	6.0	7.0	1.0
7.	**CDB20**	**6.0**	**8.0**	**1.4**
8.	**CDB22**	**10.0**	**14.0**	**1.5**
9.	CDB24	6.7	7.5	0.8
10.	**CDB25**	**9.3**	**11.0**	**1.7**
11.	**CDB27**	**5.3**	**8.0**	**2.7**
12.	**CDB30**	**8.5**	**11.0**	**2.5**
13.	CDB33	6.2	7.5	1.3
14.	**CDB34**	**7.4**	**9.0**	**1.6**

Among them 11 isolates gave the maximum ratio of clear zone diameter to colony diameter on the CMC agar plate as compared to plates cultured with the other strains. From these 14 isolates 11 isolates were efficient cellulase producers. All these 11 isolates then analyzed for secondary screening.

3.2 Secondary screening and production of cellulase enzyme:

On the basis of primary screening the potencial isolates were then evaluated for their enzyme productivity in submerge fermentation process. For the enzyme activity study, both crude enzyme and partially purified enzyme samples were assayed by cellulase enzyme assay method.

3.3 Enzyme activity assay:

The protein concentration in crude samples was determined with bovine serum albumin (BSA) as standard. The enzyme unit (EU) of both crude and partially purified enzyme was determined by using DNSA method and their specific activity was calculated which is listed in Table-3.

Table-3 specific activity of enzyme from different isolates

Isolate no.	Specific activity (U/mg of protein)	
	Crude enzyme	**Partially purified enzyme**
CDB7	4.0	4.21
CDB11	3.2	3.8
CDB14	3.8	4.0
CDB16	3.0	3.22
CDB17	4.23	4.89
CDB20	4.76	5.0
CDB22	5.0	5.34
CDB25	2.8	3.4
CDB27	**5.95**	**6.3**
CDB30	**6.0**	**6.97**
CDB34	**5.0**	**5.2**

3.4 Identification of most efficient cellulase producing bacteria:

Isolates were tentatively identified on the basis of their morphological, cultural and biochemical characteristics following Bergey's Manual of determinative bacteriology (Holt *et al*., 1994) and methods given by Cappuccino and Sherman (1993). CDB27 and CDB30 were identified to be *Pseudomonas sp*and *Bacillus sp* respectively. Their colonial, morphological, and biochemical characteristics are tabulated in Table- 4, 5.

Table- 4 Colony and morphological characteristics of most efficient isolates.

Isolates no.	Colony characteristics		Growth charecteristics on special media	Morphology
	On nutrient agar plate	**On Mac Conkey's agar plate**		
CDB27	Small, circular, entire, raised, smooth, moist, translucent, green color pigmented colonies.	Pinpoint, circular, entire, raised, moist. -Lactose non fermenter pale yellow in color	**On Cetrimide agar:** Growth observed with pin point, circular, smooth colonies.	Gram negative, short to long rods, singly arranged. Actively motile.
CDB30	Large, irregular, entire, raised, dry, creamy, opaque, non pigmented colonies.	_	**On Potato slice medium:** Creamy white dry growth observed.	Gram positive, thick long rods, arranged in a chain of 3-4 cells. Motile. Presence of central endospore

Table-5 Biochemical characteristics of isolates

Biochemical tests	CDB27	CDB30
Indole production	-	+
Catalase	+	+
Oxidase	+	+
Methyl red	+	+
Voges-Proskauer	+	+
Citrate utilization	+	+
Nitrate reduction	-	+
Phenylalanine deamination	-	-
Gelatin liquefaction	+	+
Starch hydrolysis	-	+
Casein hydrolysis	+	+
Ammonia production	+	+
Sugar fermentation		
Glucose	+	⊕
Lactose	+	⊕
Maltose	-	+
Xylose	+	⊕
Mannitol	-	⊕
Sucrose	-	-
Fructose	+	⊕
Ribulose	-	-
Arabidose	-	-

Note: (-) Negative, (+) Positive and ⊕ presence of acid and gas.

3.5 Optimization of cellulase production:

The optimum parameters were determined for cellulase production from the efficient isolates. After fermentation at the different parameters the crude enzyme product was collected for determination of enzyme activity. Enzyme activity was determined by DNSA method. The enzyme activity of CDB27 and CDB30 at the different parameters is tabulated in Table-6.

Table-6 Optimization of cellulase production.

Different parameters	Different values	Enzyme activity of CDB27 (U/ml)	Enzyme activity of CDB30 (U/ml)
Temperature	35^0C	0.98	1.2
	40^0C	**2.56**	1.89
	45^0C	2.1	2.6
	50^0C	1.86	**3.5**
	55^0C	1.11	3.0
pH	6	1.0	2.7
	7	**3.11**	3.5
	7.5	2.6	**4.9**
	8	1.11	3.7
Incubation period	48h	1.34	1.87
	72h	2.21	3.46
	96h	**3.2**	**5.7**
	120h	2.89	4.32
Substrate concentration (CMC)	0.2%	1.56	1.11
	0.5%	**3.4**	2.78
	1.0%	3.0	**5.2**
	1.5%	2.5	4.6
Inoculum size	1%	2.1	3.0
	2%	**3.5**	**4.89**
	4%	3.0	4.0
	6%	2.4	3.45

Data illustrated in Fig. (1) Clearly indicated that the highest enzyme activity of CDB27 was found to be 2.56 U/ml at 40 °C. The enzyme activity of CDB30 was gradually increased with the increase in temperature and the highest cellulase activity was found to be 3.5U/ml at 50°C.Like temperature, pH is also an important factor that influences the cellulase yield. The results illustrated by Fig. (2) Clearly show that cellulase production, expressed as enzyme activity, gradually increased as the pH values increased from 6 to 7 and reached its maximum at pH of 7 for CDB27 and 7.5 for CDB30 being 3.11 & 4.9 U/ml of cellulase respectively.The optimum value of two parameters, incubation period and inoculums size is being same for both CDB27 and CDB 30. The incubation period of 96h and the inoculum size of 2.0 % achieved the highest cellulase enzyme production which is illustrated in Fig. (3) and (5). The 0.5% concentration of substrate (Carboxy methyl cellulose) results in an increase in enzyme activity of CDB27, being 3.4 U/ml and 1% concentration of the CMC results in an increase in enzyme activity of CDB30, being 5.4 U/ml of cellulase activity.These data indicated that the isolate CDB30 *(Bacillus sp*) is a more efficient cellulase producer in comparing to CDB27 (*Pseudomonas Sp*).

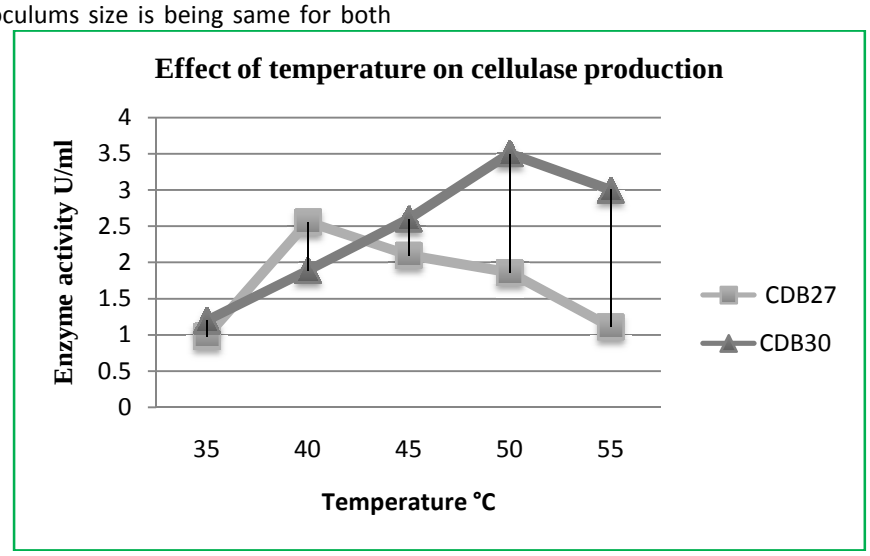

Figure-1 Effect of different different temperature on the production of cellulase by CDB27 and CDB30.

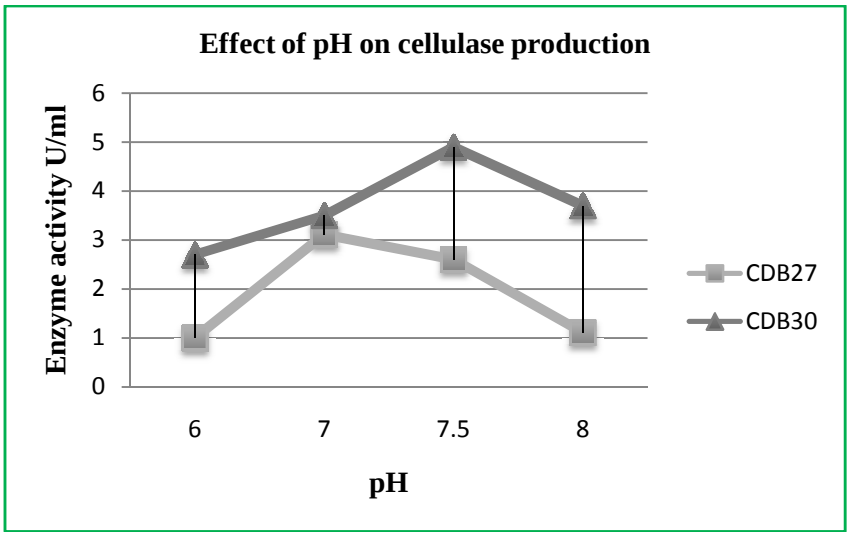

Figure-2 Effect of different different pH on the production of cellulase by CDB27 and CDB30.

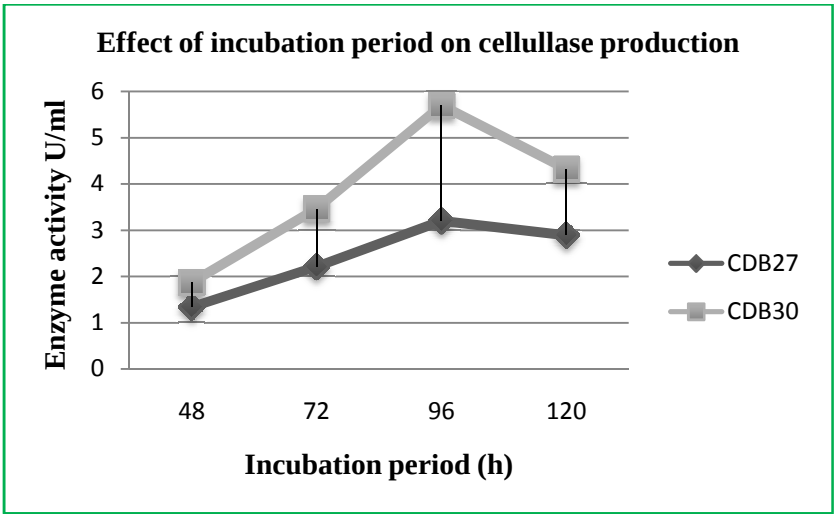

Figure-3 Effect of incubation period on the production of cellulase by CDB27 and CDB30.

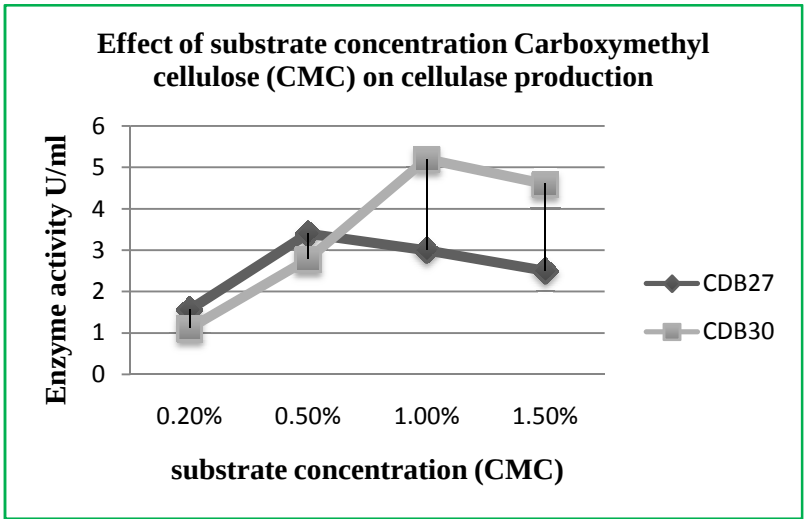

Figure-4 Effect of different concentration of Carboxymethyl cellulose on the production of cellulase by CDB27 and CDB30

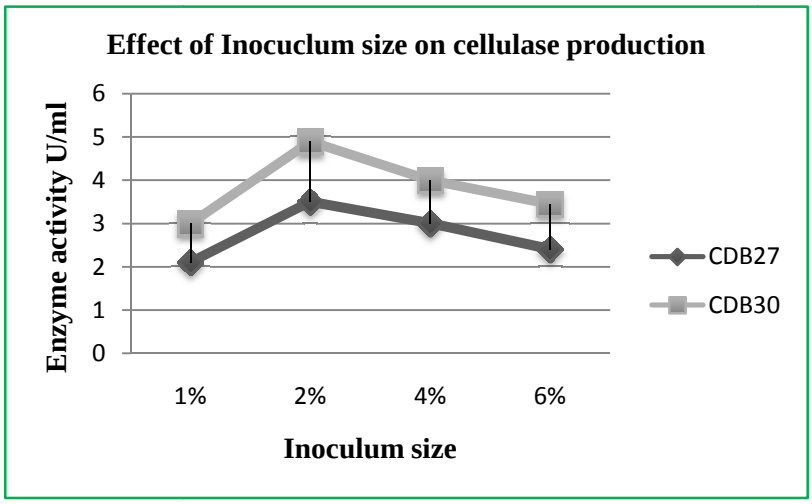

Figure-5 Effect of Inoculum size on production of cellulase by CDB27 and CDB30.

3.6 Application in biodegradation:

As CDB30 shows highest cellulase activity it was selected for filter paper degradation and cotton degradation. After continuous observation of 6 days the experiment could be concluded that CDB30 was able to degrade the filter paper but was less efficient for cotton treatment.

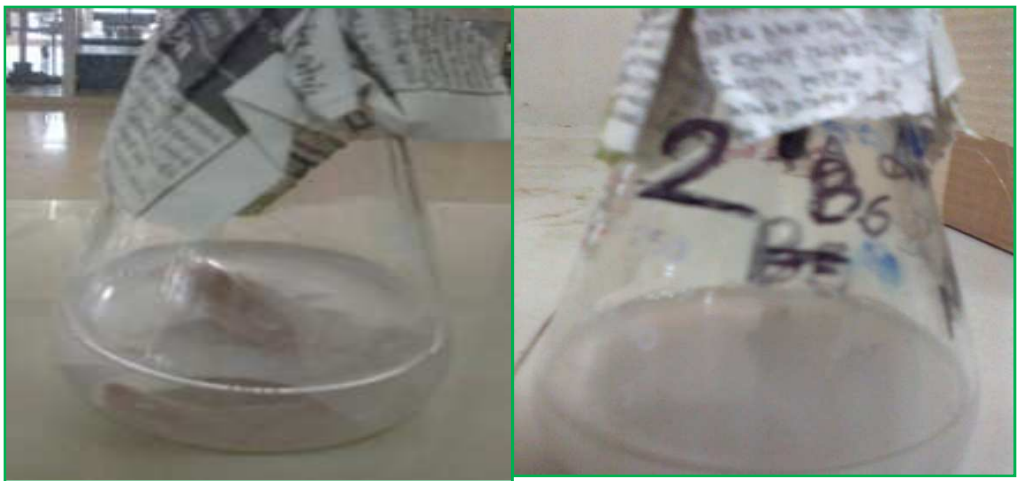

Fig-6 (a) Before incubation **Fig-6 (b) After incubation**

Figure- 6 Filter paper degradation.

Fig-7 (a) Before incubation **Fig-7 (b) After incubation.**

Figure- 7 Cotton degradation.

Degradation of cellulosic materials is a complex process requiring participation by a number of microbial enzymes. Habitats that contain these substrates are the best sources in which to find these microorganisms (Huang and Monk, 2004). So the sites for sample collections were selected as those were rich in cellulosic biomass such as wood furnishing region, paper industry waste, municipal waste and sugarcane farm, hence there were maximum possibilities to get potential cellulase producing bacterial strain. The cellulolytic activity shown by the isolated bacterial species was reported to depend on the source of occurrence in various natural environments enables them to be responsible for the degradation of cellulose that occurs in various amount of biowaste (Shankar andIsaiarasu, 2011). A rapid primary screening of isolates was carried out for their cellulase activity by using media containing 1 % CMC as a sole source of corbon and after incubation the plates were flooded with 1% Congo red dye, after 15 mins the dye was decanted and the plates were again flooded with 1M NaCl for 15 mins. The zone of hydrolysis was appear to be pale orange to clear against red background which showed the cellulase activity (Ariffin*et.al.,* 2006) (Sadhu and Maiti*.,* 2013). The secondary screening was done on the basis of their ability to show maximum enzyme activity by determination of Enzyme Unit/ml which defines as 1µmole of product release per 1 ml of enzyme per 1 min.. The extracellular cellulases produced by isolates were partially purified by ammonium sulphate precipitation and dialysis (Lee *et al.,* 2008) and the determination of total protein concentration of both crude and partially purified enzyme was carried out by Lowry method (Lowry *et al*., 1951) and their pecific activity was determined which defines as ratio of the enzymatic activity to the total amount of protein in a sample and is measured in units per mg of protein. Further, selected potent bacterial isolates were

characterized for their morphological, cultural and biochemical characteristics as describedBergey's Manual of determinative bacteriology (Holt *et al*., 1994) and methods given by Cappuccino and Sherman, CDB27 and CDB30 were identified as *Pseudomonas sp* and *Bacillus sp* respectively. The different parameters for submerge fermentation were optimized at lab scale for both isolates to improve the performance and stability of cellulase and for reucing the cost of production process. Enzyme production was tested at different pH, temperature, and incubation period, different concentration of CMC and at a different inoculums size. CMC was selected as a substrate which gave the highest yield of enzyme because it was assumed that this was due to the less complexity and hence easy assimilation of it by the isolated microbe (Wood and Bhat, 1988). The optimum temperature for cellulase production for *Pseudomanassp* and *Bacillus sp* was found to be 40^0C and 50 ^{0}C respectively; this thermostable property of cellulase has been shownthe interest of industrial applications. Optimum pH was around neutral for both the strains and the two other optimum parameters incubation temperature and inoculums size were found similar for both the strains. In compare to the strain CDB27 (*Pseudomonas sp),* CDB30 (*Bacillus sp)* show the higher enzyme activity and specific activity, so it was found to be a most efficient cellulase producer, and its natural source for the isolation was a paper industry waste. This CDB30 (*Bacillus sp)* was further applied for the biodegradation of filter paper and cotton and after the 6 day incubation it was found that the CDB30 (*Bacillus sp)* was able to degrade the filter paper completely but not the cotton, its ability to degrade filter paper represent the production of more than one type of enzyme and ability to degrade crystalline cellulose (Maki *et.al.,*2011) but it might require further incubation for cotton treatment. The efficient isolate *Bacillus sp*showed a potential to convert cellulose into reducing sugars which could be readily used in many applications such as animal foods and a feedstock for production of valuable organic compounds (Niranjane*et al.,* 2007).

4.0 Conclusion:

- The present work was carried out for isolation of potential cellulase producing bacterial strain and for that different environment which were rich in cellulosic biomass were selected as sources for isolation.
- Two isolates CDB27 and CDB30 were selected for the determination of potential cellulase activity and the source for both the isolates was paper industry waste. These CDB27 and CDB30 were characterized from their morphological, cultural and biochemical analysis and identified as *Pseudomonas sp* and *Bacillus sp* respectively.
- Partial purification of cellulase was done and the enzyme activity and specific activity was determined.
- The optimum parameters required for the stability and batter activity of enzyme were also studied.
- From the determination of enzyme activity and specific activity, the isolate CDB30 (*Bacillus sp*) was appear to be most efficientcellulase producer and also it has been shown its ability to degrade crystalline cellulose in the biodegradation study.
- Further improvement in the performance of cellulase can be imparted by mutagenesis and protein engineering techniques for the batter industrial applications.

5.0 Acknowledgement:

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