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Optimization of Culture Conditions for the Production of Extracellular Cellulase from *Corynebacterium lipophiloflavum*

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De-inking
De-sizing

Abstract

Cellulolytic enzyme producing bacteria were isolated from decaying vegetables on nutrient agar plates. As many as fourteen different bacterial strains were isolated and they were screened for the production of cellulase enzyme on a medium containing carboxymethyl cellulose (0.5% w/v). A Congo red dye based qualitative assay was performed to identify cellulase producing bacteria isolated from the vegetables. Among the difference strains, after screening on CMC agar, one strain exhibited relatively higher cellulase activity and was identified as *Cornybacterium lipophiloflavum* by a bacteriologist. Culture conditions were optimized for the above bacterium in the production medium supplemented with different carbon and nitrogen sources, different pH and different concentrations of CMC. Glucose and yeast extract proved to be the suitable carbon and nitrogen sources while pH 7.0 was ideal for cellulase production by the bacterium. Carboxymethyl cellulose at 1% in the medium induced relatively higher cellulase activity. Electrophoretic analysis of the ammonium sulfate precipitated proteins showed three prominent bands and whose molecular masses were estimated as 60, 69 and 75 kDa. Cellulase produced by *Cornybacterium lipophiloflavum* could efficiently remove dyes or inks from the pulp incubated either in the form of intact growing cell or with clarified supernatant. The enzyme also removed starch from the fabric incubated with culture supernatant suggesting the potential use of *Cornybacterium lipophiloflavum* cellulase in industrial applications.

1. Introduction

Cellulose is the most abundant polymer found on the earth. Microorganisms such as bacteria and fungi are known to produce extracellular cellulases when they grow on cellulosic materials under natural environment (Lee, 2001). The complete enzymatic hydrolysis of cellulosic material needs different types of cellulases such as endoglucanase (1, 4-α-D-glucan-4-glucanohydrolase; EC 3.2.1.1), exocellobiohydrolase (1, 4- α-D -glucan glucohydrolase; EC 3.2.1.74) and α -glucosidase (α-D -glycoside glucohydrolase (EC 3.2.1.21) (Yi, 1999). The endoglucanase randomLy hydrolyzes α -1, 4 bonds in the cellulose molecule, and the exocellobiohydrolases in most cases release a cellobiose unit showing a recurrent reaction from chain extremity. Lastly, the cellobiose is converted to glucose by α –glucosidase (Bhat, 1997). Cellulases have attracted much interest because of the diversity of their application. The major industrial applications of cellulases are in textile industry for 'bio-polishing' of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness (Cavaco-Paulo, 1998). Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juices, and in baking, while de-inking of paper is yet another emerging application. A potential challenging area where cellulases would have a central role is the bioconversion of renewable cellulosic biomass to commodity chemicals (Gong, 1999 and Himmel, 1999). Application of enzymes in detergent, leather and paper industries demand identification of highly stable enzymes active at extreme pH and temperature. The search for extremophilic organisms is one of the means for obtaining enzymes with properties suitable for industrial applications. There are quite a few advantages in using thermostable enzymes in industrial processes as compared to thermolabile enzymes (Kristjansson, 1989). The main advantage is that as the temperature of the process is increased, the rate of reaction increases. A 10 % increase in temperature approximately doubles the reaction rate, which in turn, decreases the amount of enzyme needed (Haki, 2003).

An attempt has been made in the present study to isolate, identify, and optimize cellulase producing bacteria from decaying vegetables and to evaluate its potential for industrial applications like de-inking and sizing. The results obtained in this study have been reviewed with available literature.

2. Materials and Methods

Isolation and preparation of pure cultures of bacteria

Decaying vegetables were collected from nearby vegetable markets and the decayed portions were excised and inoculated on nutrient agar plates. The agar plates were incubated at room temperature (~27ºC). Pure cultures were prepared by streak plate method and they were maintained on agar plate by sub culturing once in a week.

Screening bacteria for cellulase activity

After the pure culture formation, individual bacterium was inoculated on CMC agar medium containing carboxymethyl cellulose (5g/L), Peptone (5g/L), NaCl (5g/L), beef extract (3g/L), and agar (20g/L). The pH of the medium was adjusted to 7.0. The CMC agar plates were incubated at 37°C for 24 h. A preliminary qualitative assay for cellulolytic activity was carried out using Congo red dye. At the end of the incubation, the agar medium was flooded with an aqueous solution of Congo red (0.1% w/v) for 15 min. The excess Congo red solution was poured off, and the plates were further treated by flooding with 1M NaCl for 15 min. Congo red binds with carboxymethyl cellulose and turns into bright red. Cellulase produced by individual bacterium hydrolyzed carboxymethyl cellulose around the bacterial colony and the dye Congo red unable to stain it. Therefore the hydrolyzed zone appears transparent while the unhydrolyzed regions appear bright red. The ratio of the diameter of the clear zone to colony diameter was measured in order to select the highest cellulase producing bacterium. The largest ratio was assumed to contain the highest activity.

Identification of highest cellulase producing bacterium

The bacterium which showed the highest cellulase activity was identified by using the book of classification of bacteria form bergeys manual of determination bacteriology (Bergey, 1984).

Optimization of culture conditions for the highest cellulase production

Effect of incubation time on cellulase production

The bacterium *Cornybacterium lipophiloflavum* was incubated in the production medium consisting of the following ingredients; Glucose (5 g/L), KH_2PO_4 (1 g/L), K_2HPO_4 (11.5 g/L), $Mg_2SO_4.7H_2O$ (0.04 g /L), Yeast Extract (5 g/L), $CaCl_2.CH_2O$ (0.5g/L), $FeSO_4.7H_2O$ (0.00125 g/L), and carboxymethyl cellulose (10 g/L) with a pH of 7.0. The cultures were incubated in a shaker at room temperature (~27ºC) for different periods of time i.e., 24, 48, 72 and 96 h after incubation. The total protein content and cellulase activity were estimated as per the method described below.

Estimation of protein content and cellulase activity

The total protein content in the culture supernatant was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Cellulase activity in the culture supernatant was estimated by the method of Miller (1959). To 1.0 mL culture supernatant, 2.0 mL of CMC substrate (0.25% w/v) was added, mixed well and incubated at 500C for 5 min in a water bath. The reaction mixture was cooled under running tap water. Three milliliter of DNS reagent (1.0% v/v) was added and the tubes were boiled in a water bath for 5 min. The reaction mixture was cooled under running tap water and the absorbance was measured at 540 nm in a spectrophotometer. One enzyme unit of cellulase activity was defined as the amount of cellulase required for the hydrolysis of one µ mole of CMC in 5 min.

Effect of carbon and nitrogen sources on cellulase production

Different carbon sources such as sucrose, maltose, glucose and cellobiose and nitrogen sources such as $(NH4)_2SO_4$, peptone, yeast extract and $NaNO_3$ were incorporated at a final concentration of 0.5% (w/v) in the production medium The bacterium was grown in the medium with different carbon and nitrogen sources for 96 h at room temperature. Culture supernatant was collected and used for the estimation of total protein and cellulase activity.

Effect of pH on cellulase production

Prior to inoculation, the pH of the production medium was adjusted to 6, 6.5, 7, 7.5 and 8.5. The bacterium *Cornybacterium lipophiloflavum* was inoculated in the production medium with different pH's and incubated for 96 h at room temperature. The total protein content and amylase activity were measured as described earlier.

Effect of concentration of carboxymethyl cellulose on cellulase production

Three different concentrations of carboxymethyl cellulose i.e., 0.5, 1.0 and 1.5% were included in the production medium and the cellulase activity was estimated using the method describe earlier.

Partial purification of extracellular cellulase

The proteins in the crude culture supernatant were precipitated with ammonium sulphate at different saturation levels (60, 70 and 80% w/v). The precipitated proteins were collected by centrifugation at 7000 rpm for 15 min. The precipitate was dissolved in sodium acetate buffer

(100 mM; pH 5.5) and dialyzed against the same buffer (10 mM; pH 5.5). The dialyzed sample was lyophilized and stored at -200 C until further use.

Qualitative assay for cellulase

A carboxymethyl cellulose based agar plate method was used for qualitative analysis of cellulase produced by the bacteria. The agar plate was prepared with carboxymethyl cellulose (0.5% w/v) and agar (2% w/v). After solidification, wells (10 mm dia) were formed aseptically with the help of a cork borer. The wells were filled with either culture supernatant or lyophilized sample and incubated at 370C overnight. Negative control was maintained by adding buffer in one of the wells of the same plate. After the incubation time, the medium was flooded with Congo red dye solution (0.1% w/v). The hydrolytic zone was measured and recorded.

SDS-PAGE analysis of cellulase

Electrophoretic analysis of partially purified cellulase was performed on SDS-PAGE (12% w/v) under denaturing condition as per the protocol described by LaemmLi (1970). The proteins were stained with silver nitrate. The molecular mass of partially purified cellulase was determined by comparing the relative mobilities of standard protein markers (Genei, Bangalore).

Evaluation of *Cornybacterium lipophiloflavum* cellulase for industrial applications

Deinking potential using bacterial cells

Printed white papers with ink on it were pulped by soaking in hot water for 2 h, macerated in a domestic mixer. The macerated pulp was oven-dried at 50□ C overnight and stored in sterile container under refrigeration until further use. LB broth (50 mL) with CMC (0.1% w/v) was prepared and 1.5 g of the sterile paper pulp was mixed and inoculated with cells of *Cornybacterium lipophiloflavum*. The culture was incubated for 4 days at room temperature. Uninoculated LB broth with 1.5 g paper pulp was maintained as negative control. The deinking of the paper pulp was qualitatively assessed visually.

Deinking potential using immobilized bacterial cells

Fifty milliliters of exponentially grown cells of *Cornybacterium lipophiloflavum* was mixed with 50 mL of 2% sodium alginate solution. The mixture was added drop wise in 0.2 M CaCl2 and the beads formed were inoculated with 1.5 grams of paper pulp in a fresh medium containing CMC (0.1% w/v). The pulp with the beads was incubated for 96h at room temperature. After this, the pulp was collected by filtration through filter paper and air dried for 24 h. The pulp treated with uninoculated medium served as the control.

Deinking potential using cell-free culture filtrate

The bacterial cultures were grown in LB broth with CMC (0.1% w/v) for 96 h at room temperature. The culture was filtered through 0.45 μM filter (Nalgene, USA). The sterile culture filtrate was mixed with 1.5 grams of paper pulp and incubated at room temperature for 4 days. Uninoculated LB broth with 1.5 grams paper pulp was maintained as control.

De-sizing

Cotton fabric sized with starch was taken and tested for the presence of starch using iodine solution. Appearance of blue colour indicated the presence of starch. 1 sq. inch of fabric was cut and incubated in cell free supernatant for two days. A similar piece of fabric was incubated in sodium acetate buffer (100 mM; pH 5.5) and maintained as control. After the incubation period, the fabric was tested for the presence of starch with iodine solution.

3. Results

Isolation and screening of cellulase producing bacteria

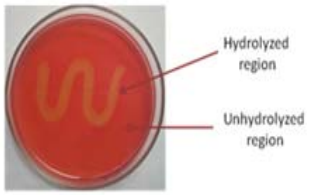
As many as 14 different bacterial strains were isolated from decaying vegetables. Pure cultures of all the fourteen strains were prepared on agar plates. All the strains were tested for cellulase activity on a medium containing CMC as the substrate. As shown in Fig.1, the cellulase produced by the bacterium hydrolyzed the substrate and the hydrolyzed regions were highly visible after staining with Congo red dye. After staining, the hydrolyzed regions appeared transparent while the unhydrolyzed regions appeared intense red. The appearance of a hydrolytic zone is a clear indication that the bacteria had produced the cellulase. After 24 h of incubation, all strains of the fourteen bacteria showed signs of growth on CMC agar but only one strain hydrolyzed the CMC very efficiently. The bacterium was identified as *Cornybacterium lipophiloflavum* by using the book of classification of bacteria form bergeys manual of determination bacteriology. Since the sole carbon source in the medium was CMC and the strong hydrolytic activity shown by the bacterium is a clear evidence for the production of cellulase.

Screening for cellulolytic bacteria

Preliminary screening of cellulase producing bacteria was carried out based on a Congo red dye based method as described in the material and methods. After 24 h incubation, all the fourteen strains of bacteria showed signs of growth on CMC agar however only one bacterium was able to hydrolyze CMC incorporated in the medium very

efficiently. This particular bacterium was chosen for identification and for further experiments. The bacterium was identified as *Cornybacterium lipophiloflavum* by using the book of **classification of bacteria form bergeys manual of determination bacteriology.** Since the sole carbon source in CMC agar was CMC (cellulose), therefore the result obtained here is a strong evidence for the production of cellulase (Fig.1).

Figure: 1 screening for cellulolytic bacteria on carboxymethyl cellulose agar plate

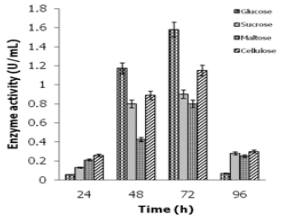


Optimization culture conditions for cellulase production

Effect of carbon sources on cellulase production

Carbon sources such as glucose, sucrose, maltose and cellobiose at a final concentration of 0.5% (w/v) was incorporated in the medium and the bacterium was grown for different time periods like 24, 48, 72 and 96 h. The bacterium, *Cornybacterium lipophiloflavum* produced the highest amount (14.1 U/ mL) of cellulase enzyme in the medium incorporated with glucose at 72 h after incubation (Fig.2) followed by medium incorporated with cellobiose and sucrose. Maltose induced the least amount of cellulase activity among the carbon sources used (0.72 U/mL; Fig.2). The highest production of cellulase was observed on 72 h after incubation. The amount of enzyme activity decreased when the incubation time is decreased or increased from 72 h.

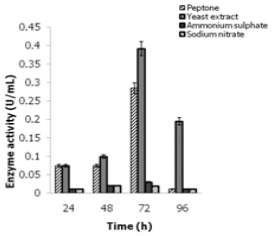
Figure: 2 Effect of carbon sources on cellulase production



Effect of nitrogen sources on cellulase production

Different nitrogen sources such as peptone, yeast extract, ammonium sulphate and sodium nitrate were incorporated in the medium at a final concentration of 0.5% (w/v) and the bacterium was incubated for 24, 48, 72 and 96 h. As shown in Fig. 3, the bacterium produced the highest level of cellulase in the medium incorporated with yeast extract followed by peptone (0.392 U/mL and 0.27 U/mL; Fig.3). Ammonium sulfate and sodium nitrate did not induce the enzyme production at all. The production of cellulase nitrogen source amended medium is more pronounced at 72h and it declined if the incubation time is either decreased or increased from 72 h (Fig.3).

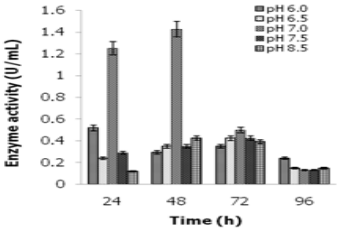
Figure: 3 Effect of nitrogen sources on cellulase production



Effect of pH on cellulase production

The organism produced the highest cellulase enzyme in the medium incubated at pH 7.0 (0.80 U/mL; Fig. 4) when compared to the medium maintained at other pH's i.e., 6, 6.5, 7.5 and 8.5. There was a drastic decrease in the enzyme activity when the organism was grown in medium whose pH was adjusted to either below or above pH 7.0. The organism produced the highest cellulase when incubated for 48 h (Fig. 4) followed by 24 h. Production of cellulase drastically reduced when the organism was incubated at 72 and 96 h.

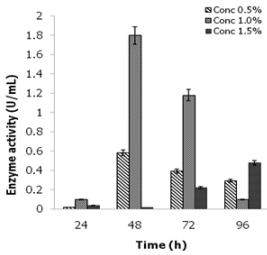
Figure: 4 Effect of pH on cellulase production



Effect of substrate concentration on cellulase production

The bacterium, *Cornybacterium lipophiloflavum* was grown in the medium containing different concentrations of carboxymethyl cellulose i.e., 0.5, 1.0 and 1.5% (w/v) for 24, 48, 72, and 96 h. Cellulase activity was measured from the culture supernatant. As shown in Fig.5, the highest cellulase activity (1.15 U/mL) was observed in medium incorporated with 1% CMC followed by 0.5 and 1.5% (w/v). The highest cellulase activity (1.15 U/mL) was measured on 48 h followed by 72 h. The least cellulase activity was measured at 24 h (0.24 U/mL).

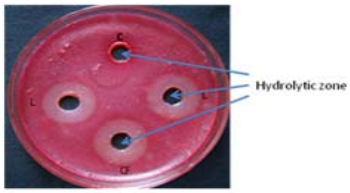
Figure: 5 Effect of substrate concentration on cellulase production



Qualitative assay for the detection of cellulase activity

The cell free culture supernatant and ammonium sulfate precipitated proteins were tested for cellulase activity qualitatively using CMC agar plate assay as described in material and methods. Just after solidification of the medium, wells (10 mm dia) were formed with the help of cork borer. The wells were filled with culture supernatant and ammonium sulfate precipitated samples and incubated overnight at room temperature. Congo red dye solution was layered on the agar and observed for hydrolytic zone around the well. As shown in Fig. 6, the wells filled with both culture supernatant and ammonium sulfate precipitated samples hydrolyzed the CMC around the wells (arrows) whereas the well filled with uninoculated medium which served as the control did not show hydrolytic activity around the well.

Figure: 6 Qualitative assay for cellulase activity

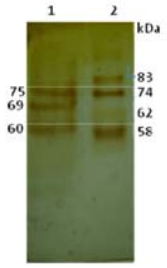


C-Control; CF-culture filtrate; L-Ammonium sulfate precipitated protein

Electrophoretic analysis of partially purified cellulase on SDS-PAGE

The ammonium sulfate precipitated proteins were electrophoreses on polyacrylamide gel (12%; w/v) and stained with silver nitrate. As shown in Fig. 7, there were three prominent bands appeared on the gel after staining (Fig.7; lane 1). The approximate molecular masses of these proteins were determined to be 60, 69 and 75 kDa (from bottom to top).

Figure: 7 SDS- PAGE analysis of ammonium sulfate precipitated proteins



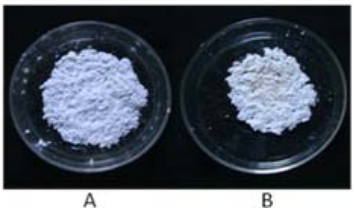
Lanes, 1- Ammonium sulfate precipitated proteins; 2. Protein markers

Analysis of *Cornybacterium lipophiloflavum* cellulase for industrial applications

Deinking potential using bacterial cells

Macerated paper pulp was incubated with growing cells in LB broth for 96 h and the decolourization of the paper pulp was examined. As shown in Fig. 8, the pulp incubated with growing cells of *Cornybacterium lipophiloflavum* decolorized the ink to certain extent while the pulp incubated with uninoculated medium did not decolorize (Fig.8).

Figure: 8 Analysis of de-inking potential of *Cornybacterium lipophiloflavum* growing cells with pulp



A-control, B-Pulp treated with bacterial cells

Deinking trials using cell-free bacterial culture filtrate

As evident from Fig. 9, the paper pulp treated with culture supernatant decolorized the pulp completely and appeared bright white while the pulp treated with uninoculated medium remained blue. The decolorization was much more effective in the supernatant supplemented with CMC.

Figure: 9 Analysis of de-inking potential of *Cornybacterium lipophiloflavum* culture supernatant with paper pulp

A-Control (pulp treated with uninoculated medium)
B-Treated (pulp treated with culture supernatant)

Deinking trials using immobilized bacterial cells

In order to analyze the decolourizing potentials of Cornybacterium lipophiloflavum, the cells were immobilized in sodium alginate beads and incubated with paper pulp (1.5% w/v) in LB medium for 96 h. As shown in Fig.10, the immobilized cells could decolorize the pulp completely.

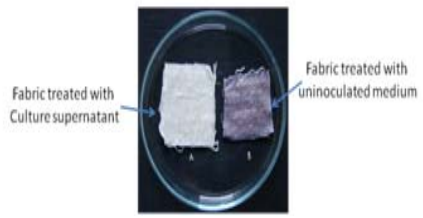
Figure: 10 De-inking potential of immobilized cells of *Cornybacterium lipophiloflavum* with paper pulp

A-Control (pulp treated with uninoculated medium
B – Treated (pulp treated with immobilized cells)

Analysis of de-sizing potential of cellulase of *Cornybacterium lipophiloflavum*

The cotton fabric with starch on it was incubated with cell-free supernatant for 48 h and analyzed for de-sizing. After 48 h treatment, the fabric was treated with 1% iodine solution which acts as an indicator for the presence of starch. As shown in Fig.11, the fabric treated with the culture supernatant completely removed the starch and appeared clear white whereas the untreated fabric stained blue.

Figure: 11 Analysis of *Cornybacterium lipophiloflavum* culture supernatant for de-sizing of fabric



4. Discussion

A preliminary investigation has been made in this study to isolate, identify and screen for cellulolytic enzyme producing bacteria which were isolated from decaying vegetables. Attempts have also been made to optimize the culture conditions for the optimal production of cellulase using the promising strain of bacteria and evaluated for industrial applications like de-inking of paper pulp and de-sizing of fabric. It has been planned to isolate the cellulolytic bacteria from decaying vegetables because vegetables are the chief source of cellulose. Therefore, cellulose degrading bacterium would normally dwindle the decaying vegetables. After the bacterial isolation each of the bacterial strains were analyzed for its potential to produce cellulolytic enzyme employing a simple qualitative Congo red dye based method. The samples containing bacteria were inoculated on a medium containing CMC (0.5% w/v). The plates were incubated overnight at room temperature and the medium was flooded with Congo red dye solution. As is evident from Fig.1, cellulase produced by bacteria hydrolyzed the substrate (CMC) incorporated in the medium and the dye Congo red did not stain the region where the CMC is hydrolyzed. Congo red stained the unhydrolyzed CMC incorporated in the medium. Hydrolysis of CMC clearly suggests that bacteria produced cellulase outside the cells. The hydrolyzed regions appear somewhat transparent (Fig.1). A total number of 14 bacterial strains were isolated from decaying vegetables on nutrient agar plate. Of the 14 isolates only one strain potentially hydrolyzed CMC in the medium. Therefore, this particular bacterial strain was chosen and identified as *Cornybacterium lipophiloflavum* and maintained in agar slants.

The promising bacterium which showed cellulase activity was used to optimize the culture conditions for maximum cellulase production. Different carbon and nitrogen sources, different pH's and different substrate (CMC) concentrations were used for cellulase production in the medium described in material and methods. The highest cellulase activity (14.1 U/ mL) was observed in the production medium amended with 0.5% (w/v) glucose followed by cellobiose (Fig.2). It is interesting to note that the maximal cellulase production occurred 72 h and the enzyme activity decreased if the incubation time is either increased or decreased. It is obvious that at least 72 h incubation is required for efficient production of cellulase by this particular bacterium. Previous observation with *Rhizoctonia bataticola* (Goel, 1973). *Clostridium acetobutylicum* (Ait, 1979), *Alternaria alternate* (Macris, 1984), *Acetobacter* spA9 (Hong, 2001) and *Gluconaacetobacter xylinus* (Sherif, 2005), showed that glucose as carbon source in the medium induced higher cellulolytic activity in these organisms.

Among the different nitrogen sources used the highest cellulase activity was observed in medium amended with yeast extract (0.392 U/mL) which was closely followed by peptone (Fig. 3). Though yeast extract induced cellulolytic enzyme the highest cellulase activity was observed when the bacterium was grown for 72 h. Both ammonium sulfate and sodium nitrate did not induce cellulase

even as they were incubated for 72 h. These results suggest that the organism preferentially utilize yeast extract to get the nitrogen from it. *Clostridium acetobutylicum, Alternaria alternate, Acetobacter* spA9, *Gluconaacetobacter xylinus* produced cellulases when they were grown in medium supplemented with yeast extract (Macris, 1984, Allcock, 1981, Hong, 2001and Sherif, 2005).

The bacterium, *Cornybacterium lipophiloflavum* produce the maximum cellulase activity (0.80 U/mL) when the pH of the medium was adjusted to 7.0 (Fig.4). There was a drastic decrease in cellulase activity when the pH of the medium is either increased or decreased from 7.0. An interesting observation is that at pH 7.0 the highest cellulase activity occurred at 48 h which was contrary to the observations made with carbon and nitrogen sources (72 h). The cellulase activity was drastically reduced when the pH of the medium is raised to 8.5 from 7.0 which clearly indicate that this particular bacterium prefers neutral pH for its cellulolytic actions. High level of cellulolytic activity has been observed in organisms such as *Clostridium thermocellum* (Ait, 1979), *Sinorhizobium fredii* (Chen, 2004), *Gluconaacetobacter xylinus* (Sherif, 2005), *Bacillus circulans* (Ray, 2007), *Paenibacillus* sp (Emtiazi, 2007) and *Acetobacter* sp 4B-2 (Pourramezan, 2009).

As evident from Fig. 5, the test organism produced relatively higher cellulase enzyme when incubated with carboxymethyl cellulose at 1.0% followed by 0.5% (w/v). Although the maximum induction of the enzyme occurred at 48 h, a significant decrease in cellulase activity was observed when the concentration of the substrate is either increased or decreased (Fig.5). This observation suggests that scaling up of cellulase production by this organism could be done with 1% CMC concentration.

Partial purification of cellulase from the culture supernatant was attempted by selectively precipitating the enzymic proteins with ammonium sulphate. Among the three different saturations used, the proteins precipitated with 80% (w/v) showed relatively higher cellulase activity. Electrophoretic analysis of the partially purified protein on SDS-PAGE indicated three prominent protein bands whose molecular masses were 60, 69 and 75 kDa (Fig.7). Zymogram or gel activity staining analysis is required to verify if all the three proteins are isoforms of cellulase or they have different function.

The cellulase produced by *Cornybacterium lipophiloflavum* was evaluated for its de-inking and de-sizing potentials. Macerated papers with printed ink on it were incubated with intact growing cells the completely de-inked the pulp (Fig.8). Similarly, the inked pulp was incubated with culture supernatant again and observed that the pulp was de-inked which suggests that cellulase produced by *Cornybacterium lipophiloflavum* could be used for de-inking process**.** In an effort to produce sodium alginate immobilized cells with de-inking capability, the macerated pulp was incubated with sodium alginate beads and at the end of the incubation the pulp was completely free of ink. Attempt to find out the potentiality of the cellulase in de-sizing was also made by incubating the culture supernatant with sized fabrics. The fabric treated with culture supernatant completely removed the starch from it while the fabric treated with uninoculated medium did not remove the starch. This suggests that the cellulase produced by *Cornybacterium lipophiloflavum* could be very well used in industrial applications**.**

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