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Bioprocessing of Algal Waste for Cellulase Production by *Cellulomonas uda (NCIM 2353)*

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ABSTRACT:

Production of cellulase under solid state fermentation by Cellulomonas uda (NCIM 2353) has been investigated. Effect of various parameters like particles size of substrate, % moisture, pH, yeast extract content, peptone content were studied for optimization of process and better productivity Enzyme. The result presented in this study revealed that higher cellulose activity of 2.63 IU/min was achieved at 0.32 mm particle size at incubation temperature 37^0c and 12 days of incubation period. while the process optimization study indicated the enzyme production of 1.59 IU/min with moisture content 100 %, 2.43 IU/min with pH 6, 1.85 IU/min with 1.5 % w/v of yeast extract and 1.81 IU/min with 2 % w/v peptone. Results indicate the excellent scope of utilizing algal waste as solid substrate for commercial production of cellulase employing bacterial strain.

Key words: *Solid state fermentation, Cellulomonas uda, Cellulose, Yeast extract, Peptone.*

I. INTRODUCTION

Cellulase production is the most important step in the economical production of ethanol, single cell protein and other chemicals from renewable cellulosic materials. Till date the production of cellulase has been widely studied in submerged culture processes, but the relatively high cost of enzyme production has hindered the industrial application of cellulose bioconversion [1]. It has been reported that solid-state fermentation (SSF) is an attractive process to produce cellulase economically due to its lower capital investment and lower operating expenses [2]. SSF can be defined as the cultivation of microorganisms on solid substrates devoid of or deficient in free water [3]. SSF has several advantages over more conventional submerged fermentation [4]. Unfortunately, very few of these processes enter commercial production due to the magnitude of the technical difficulties in operating and optimizing large scale SSF bioreactors [4]. In

recent years, much work has been carried out towards efficient utilization of agro-industrial residues to produce enzymes of commercial importance by microorganism [5] [6] [7] [8]. *Cellulomonas uda* is one of the most important microorganisms used in industry, allows a relatively higher enzyme production of cellulase. It was reported by many authors that *Cellulomonas uda* is capable of increase the production of cellulase from substrate like algal biomass and similar others. The objective of this study was to evaluate the potential of algal biomass as a substrate for the production cellulase, using a bacterial strain of *Cellulomonas uda* in SSF.

II. MATERIALS AND METHODS

2.1. Microorganism

Cellulomonas uda NCIM 2353 was obtained from National Chemical Laboratory (NCL, Pune) a division of National Center for Industrial Microorganism (NCIM) and was maintained on nutrient agar (HiMedia, India) slant of pH 7 at 40°C. Microorganisms were subculture every week at departmental laboratory and preserved at 4°C.

2.2. Substrate preparation

Algae used as substrate was collected from water bodies of Jalgaon and sun dried for 2-3 days depending upon light conditions and moisture remained. Sun dried algae were oven dried at 60-70°C in hot air oven for 2-3 hrs. Oven dried substrate was grinded into smaller particle size by grinder. 10 ml of salt solution (yeast extract 0.3, peptone 0.5, NaCl 1.5, $Na_2HPO_4.2H_2O$ 1.1, NaH_2PO_4 0.61, KCl 0.3 and $MgSO_4.7H_2O$ 0.01) was added into 10 gm of grinded substrate. Substrate was autoclave and sterilized at 121°C (15 psi).

2.3. Inoculums preparation

The loop full of microorganisms were transferred aseptically to 100 ml conical flask containing 50 ml of sterilized inoculums medium (sterilized at 121°C for 15 minutes) containing g/100ml: glucose 2, yeast extract 0.3, peptone 0.5, NaCl 1.5, $Na_2HPO_4.2H_2O$ 1.1, NaH_2PO_4 0.61, KCl 0.3, K_2HPO_4 29, $FeSO_4$ 0.1, $MnSO_4$ 0.1, KH_2PO_4 1.8 and $MgSO_4.7H_2O$ 0.01 in laminar air flow. The flask was kept in incubator at 37°C for 48 hrs. Spores suspension 10^6–10^7 spores/ml were used as inoculums.

2.4. Solid state fermentation

All the treatments were run in duplicate. Substrate was cooled to ambient temperature. Substrate of 10 gm in petriplates and 15 gm in conical flask of 250 ml was added with the inoculum of 30 % (W/V) in aseptic conditions. The flask and petriplates were incubated at 37^0C for 2 days. The SSF media flasks and petriplates were gently shaken after every 12 hrs for uniform mixing of the substrate and microorganism.

2.5. Enzyme extraction

The enzyme was extracted by a simple contact method. The fermented algae waste sample was extracted with 1:10 (W/V) of 0.1 M sodium citrate buffer of pH 4.8 for 120 minutes at 250 rpm. The material was filtered through muslin cloth. Filtrate collected was centrifuged at 10000 rpm for 10 minutes at room temperature. Supernatant was carefully collected and use as crude enzyme extract for determining cellulase activity [9].

2.6. Enzyme assay

Cellulase enzyme activity was assayed by Cellulase activity against filter paper (FPase) method of Ray et al., [11]. Cellulase acts on cellulose to produce reducing sugar (glucose) and assayed following the method of Bernfeld [10] using 3, 5-dinitrosalicylic acid. The absorbance was measured at 575 nm. One unit of enzyme activity was defined as the amount of enzyme that release 1mmole of reducing sugar as glucose per minute under the assay condition specified.

III. RESULTS AND DISCUSSIONS

3.1. Effect of particle size on enzyme activity

The algal waste of 8 different particle sizes was used to prepare growth medium (0.11mm to 1.70 mm). The fermentation was carried out at 100 %

moisture level, and salt solution. The results are shown in Fig. 1. The maximum cellulase production was obtained at particle size 0.32, 0.46 mm. the rate of cellulase production was also maximal at 1.7 mm particle size. The lowest cellulase production was obtained at 1.31 mm particles might be due to very larger particle of the medium reduces substrate availability to microbe, resulting in low cellulase production. Cellulase production at particle size 1.7 mm is very close to the 0.32 and 0.46 mm. This presumably due to medium containing larger particle size exhibiting high porosity and hence resulting in better heat and mass transfer, which increased cellulase production. The optimum particle size of the medium was 0.32 mm which was selected for the further studies.

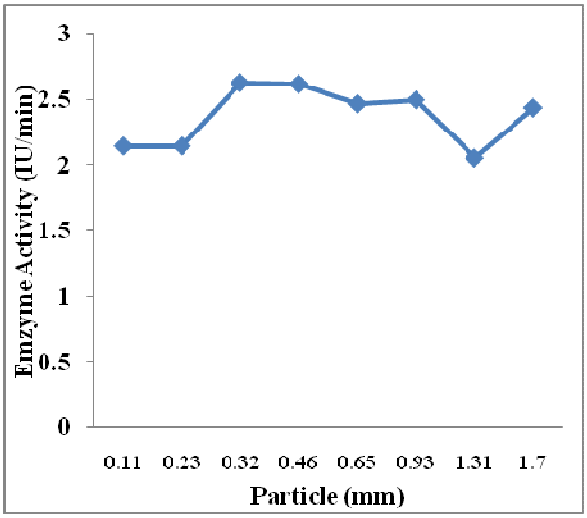

Fig: 1. Effect of particle size on enzyme activity

3.2. Effect of extraction pH on enzyme activity

Effect of extraction pHs on enzyme activity was carried out at previously optimized conditions shown in Fig. 2. The optimum pH for cellulase production was found to be 7.0. All microorganisms hold a pH range for its growth and survival with optimum value between 6 to 8 pH ranges. Initial pH influences many enzymatic systems and the transport of several species of enzymes across the cell membrane [14]. More acidic or basic pH leads reduction in utilization of nutrients in solid substrates. Neutral pH of ssf system is most effective in maximum yield.

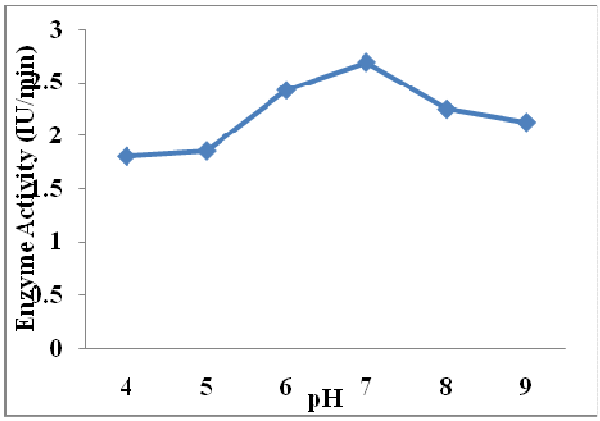

Fig: 2. Effect of pH on enzyme activity

3.3. Effect of incubation period on enzyme activity

Incubation period is considered one of the most important factors affecting cellulase production. Solid state fermentation was performed by varying incubation period from 2 to 14 days at 37°C. Data obtained from study summarize that increase in incubation period from 2 to 12 days increases enzyme activity with maximum activity of 1.97 IU/min for 12 days. However drastic decreases cellulase activity was observed at 14 days of incubation due to decreased in moisture content in substrate. The highest CMCase activity had been recorded after 7 days for *A. terreus* [15].

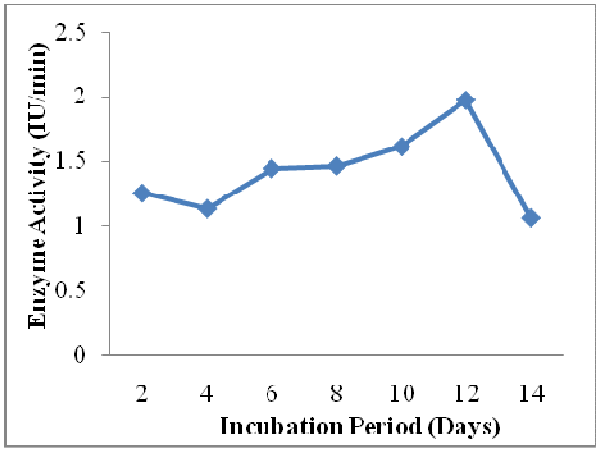

Fig: 3. Effect of incubation period on enzyme activity

3.4. Effect of moisture content on enzyme activity

The experiment was carried out at different moisture level viz. 70-140 % and particle size 0.32 mm with sodium salt. The data shown in Fig. 4 gives the effect of moisture content on cellulase production. The maximum enzyme activity of 1.59 IU/min was observed at 100% moisture content of the substrate (Fig 4). The results indicated that when moisture level increased beyond a certain limit the enzyme activity started decreasing. This decline may be due to poor aeration in SSF and partial adsorption of enzyme to the substrate. The study conducted by Xia *et al*. (1999) showed that water content of solid substrate is one of the key factors in cellulase production by solid state fermentation on lignocellulosic waste [13]. 100% moisture content was found to be the most suitable for cellulase production.

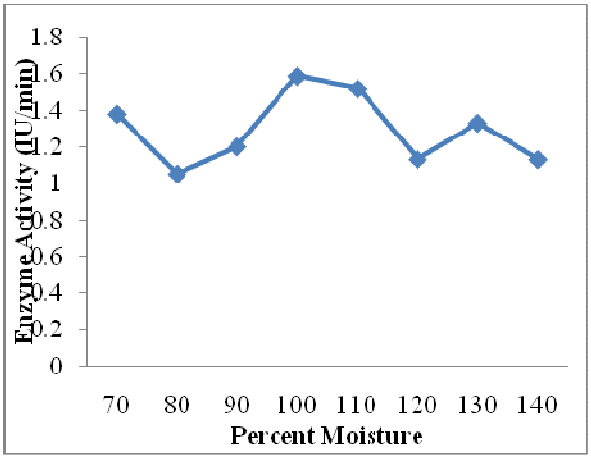

Fig: 4. Effect of moisture content on enzyme activity

3.5. Effect of yeast extract content on enzyme activity

In order to optimize yeast extract concentration for production of cellulase different concentrations of yeast extract were optimized under same conditions ranging from (0.3-1.8 gm). Enzyme activity increased with the increase in substrate concentration. It was observed that maximum cellulase activity (1.85 IU/min) was obtained at 1.5 gm yeast extract concentration (Fig. 5). Minimum yeast extract concentration results in an increase of the product yield and rate of the hydrolysis reaction. However, high yeast extract concentration might be cause substrate inhibition, which substantially lowers enzyme production.

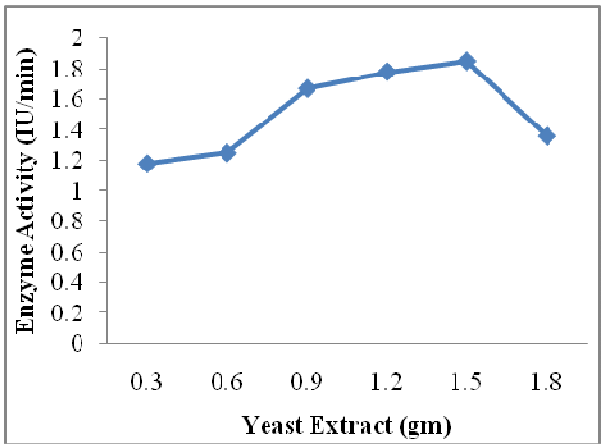

Fig: 5. Effect of yeast extract content on enzyme activity

3.6. Effect of peptone content on enzyme activity

Production of hydrolytic enzymes can be enhanced by the additional nitrogen sources like (NH4)2SO4, peptone and yeast extract. Peptone was the best nitrogen source for the cellulase production. The results obtained from the experiment reveal that, 2 gm of peptone concentration gives highest cellulase yield. On the other hand cellulase production is nearly same for 0.5-1.4 gm and decreases drastically at 1.7 gm of peptone concentration.

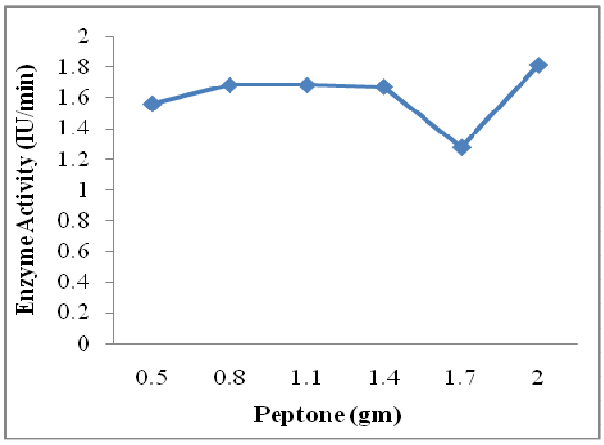

Fig: 6. Effect of peptone content on enzyme activity

[IV] CONCLUSION

The results obtained in the present study indicated *Cellulomonas uda* (NCIM 2353) as a potential strain for cellulase production using solid-state fermentation with algal waste as substrate. In accordance with the results, taking all the influencing factors and the results into consideration, the optimal cultural process was considered as follows: The optimum activity of enzyme 2.63 IU/min was obtained for 0.32 mm particle size, 37ºC incubation temperature and 12 days incubation period with extraction pH 7. Furthermore the enzyme was found to show optimum activity with 100 % moisture content, 1.5 yeast extract and 2 gm of peptone content. Above data makes the cellulase of organism useful for the various industrial applications like pulp and paper industries, textile industries and food and feed industry.

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