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Production and optimization of cellulase enzyme by *Pseudomonas aeruginosa* MTCC 4643 using sawdust as a substrate

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Abstract- The main objective of the study is to explore easier and cost effective method to produce the cellulase enzyme by using sawdust as a substrate, which is an industrial waste. In the present investigation, different cultural conditions (Concentration of sawdust, pH, Temperature and Inoculum size) were examined to assess their effect for optimum cellulase production using *Pseudomonas aeruginosa* MTCC 4643. Pretreated sawdust produced highest cellulase activity (12.08 U/g)at 5% concentration. Optimum pH and temperature for cellulase production was observed 6.0 and 30^{0}C respectively.

Index Terms- Cellulase, Lignocelluloses, *Pseudomonas aeruginosa,* Sawdust

I. INTRODUCTION

In recent years, growing attention has been devoted to the bioconversion of lignocellulosic materials to energy.Several researches have shown that the production cost of ethanol is tightly associated with the production of cellulase enzyme.Accountable studies were carried out to produce ethanol by a wide range of cellulase producing microorganisms using different types of lignocellulosic material as a substrate including bacteria such as *Pseudomonas* sp. from CMC (Bakare *et al.,* 2005), *Cellulomonas* sp. from CMC, cellulose, *Acacia auriculiformis Cunn.'s* leaves and sugar cane (Siddiqui *et al.,*1997; Rajoka, 2004 and Sankharak *et al.,* 2011) , *Bacillus* sp. from CMC, coir wastes and saw dust (Ariffin *et al.,* 2006; Verma *et al.,* 2012 and Shanmugapriya *et al.,* 2012).
In this present study, Separate Hydrolysis and Fermentation (SHF) method was adopted and sawdust was used as a carbon source under different environmental parameters (i.e., Concentration of saw dust, pH, Temperature, Inoculum size). The objective was to optimize the enzymatic hydrolysis conditions and maximize enzyme production.

II. MATERIALS AND METHODS

Substrate:Sawdust was used as a carbon source collected aseptically from M/S Sharma Hardware Pvt. Ltd., District Alwar, Rajasthan, India-301001 and sun dried to reduce the moisture content.

Pretreatment of Substrate: Sawdust was soaked in 1% sodium hydroxide solution (NaOH) in the ratio 1:10 (sawdust: solution) for two hours at room temperature and autoclaved at 121^{0}C for one hour. The treated sawdust was then filtered and washed with distill water until the washed water become neutral **(**Soloman *et al.,* 1999 and G. Immanuel *et al.,* 2007)and then dried at 50^{0}C for overnight.

Microorganism:*Pseudomonas aeruginosa* MTCC 4643**,**used for the present study was procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Culture condition and Inoculum Preparation:The bacterial culture received in vials from MTCC, Chandigarh was mixed into 1 ml saline solution and vortexed for 20 seconds. *P.aeruginosa*was sub cultured on Nutrient Agar (NA) plates at 30^{o}C for two days and stored thereafter in refrigerator at 4ºC till further use. Inoculum for bacterium was prepared in Nutrient Broth. About 100 ml of inoculum was prepared for bacterial culture in 250 ml Erlenmeyer flask. Few colonies were picked up from two days old culture and were inoculated at 30ºC on a rotary shaker (200rpm) for twenty four hours, before it was used for the saccharification process.

Minimum Culture Medium Preparation: The media contained following chemicals (g/l) in distilled water:$NaNO_3$ (4.0), $NaHPO_4$ (3.0), KH_2Po_4 (3.0), $CaCl_2$. $2H_2O$ (0.1), $FeSO_4.7H_2O$(0.001), NaCl (1.0), KCl (1.0). The pH of culture medium was set as 7.2±0.2.

Saccharification of substrate: 100 ml of the media was taken in 250 ml Erlenmeyer flask and sterilized by autoclaving 121°C for 15 min and cooled. Autoclaved sawdust was added in flask before inoculation then inoculated with 2ml of inoculum of *P. aeruginosa* MTCC 4643, under controlled conditions and incubated at 30ºC for twenty four hours. This culture was harvested after 24 hours by centrifugation at 5000 rpm for 10 min at 4°C using refrigerated ultracentrifuge. The supernatant was used as the crude extracellular enzyme source. Three replicates were set for each treatment.

Cellulase assay: A reactive mixture contains supernatant (1 ml) to 1 ml volume of CMC substrate solution. We mixed the resulting solution thoroughly and transfer to a water-bath maintained at 40.0 ± 0.1°C then after 10 minutes (reaction step),we removed the test tube from the water bath and add 4 ml of DNS-Lactose solution and mix to stop the enzymatic reaction. We covered the tubes and placed in a boiling water bath for 15 min and cooled to room temperature with a cooling water bath

and then it was used for measuring optical density at 540 nm against water blank and standard graph was made (Ghose, 1987).

Optimization of substrate concentration: To study the effect of saw dust concentration, the minimal culture medium was prepared in 250 ml conical flasks by setting the different concentrations of saw dust such as 1%, 2%, 3%, 4% and 5% respectively in triplet. The pH of minimal culture medium was set as 7.2±0.2. About 2 ml of 24 h old inoculum suspension was inoculated and placed at 30 ^{0}C for 24 h. The initial saw dust concentration that was efficiently utilized by microbe was observed and the same concentration of saw dust was used for setting experiments for optimization of other factors.

Optimization of pH: Minimal culture medium was prepared and pH was set at different level such as 5.0, 6.0, 7.0, 8.0 and 9.0 by adding 1% NaOH and concentrated HClrespectively, were tested for saccharification using initial saw dust concentration.About 2 ml of 24 h old inoculum suspension was inoculated and placed at 30 ^{0}C for 24 h.

Optimization of temperature:To optimize the saccharification temperature, saccharification was carried out at 25, 30, 35, 40 and 45ºC.

Optimization of inoculum size: To study the effect of inoculum sizes on saccharification process, the pH of minimal culture medium was set 7.2±0.2 and using the 24 h old inoculum was inoculated, by setting the different sizes of inoculum such as 1%, 2%, 3%, 5% and 10% respectively in triplet and the all flasks were placed in an incubator at 30 ^{0}C.

III. Results and Discussion

A. *Optimization of substrate concentration:*

The effect of various concentrations of substrate (1-5%) on cellulase activity in the presence of bacteria *Pseudomonas aeruginosa*MTCC 4643 is shown in Figure 1. The maximum cellulase activity (12.08 U/g) was found at 5% concentration of saw dust at temperature of 30^{0}C and pH of 7.2± 0.2.Shabeb *et al.*(2010) reported that *Bacillus subtilis* KO exhibited maximum activity at 10% concentration of molasses. Similarly Harchand and Singh (1997) investigated that *S. albaduncus*showed highest level of cellulase activity with 3% concentration of cotton used as a substrate.

B.*Optimization of pH:*

The effect of pH-value by *Pseudomonas aeruginosa*MTCC 4643 on cellulase activity was examined at various pH values ranging from 5-0 to 9.0 as shown in Figure 2. The cellulase activity was found at a broad range of pH values (pH 5-0 to 9.0) with optimal pH of 6.0(19.99 U/g). Cellulase activitywas reduced to 49% at pH 9.0.This result was approximately in correlation with the findings of many other workers. Bakare *et al.* (2005) found pH 6.5-7.0 optimum for the production using the CMC as a substrate by *Pseudomonas fluorescens.*Shankar andIsaiarasu (2011) and Ariffin *et al.* (2006) revealed that *Bacillus pumilus* produced maximum cellulase at pH 6.0.

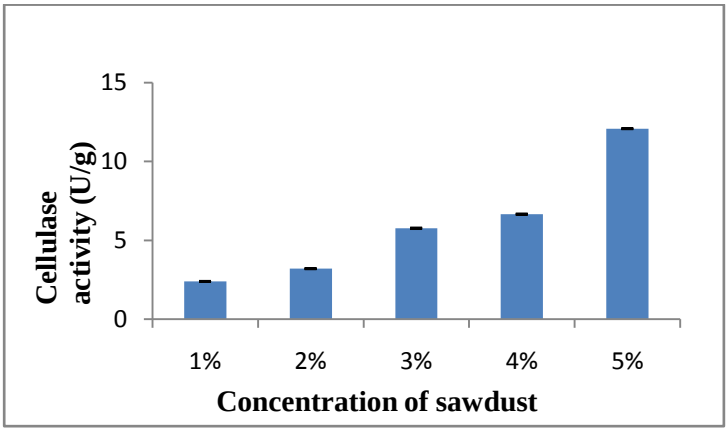

Figure1: Impact of concentration of sawdust on cellulase activity (U/g) at pH7.0±0.2; 30 ^{0}C.

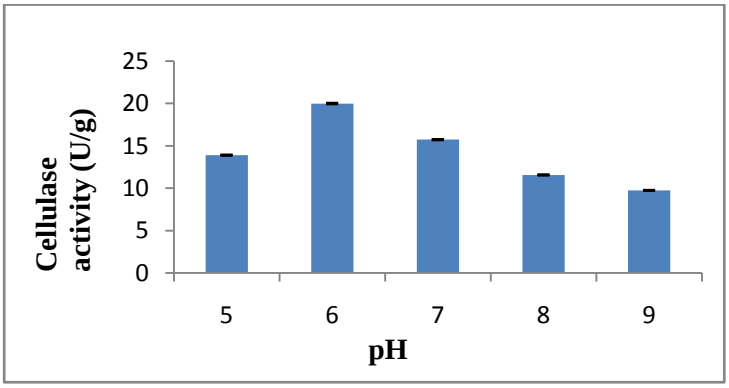

Figure 2: Impact ofpH on cellulase activity (U/g) at 30 ^{0}C using 5% substrate concentration.

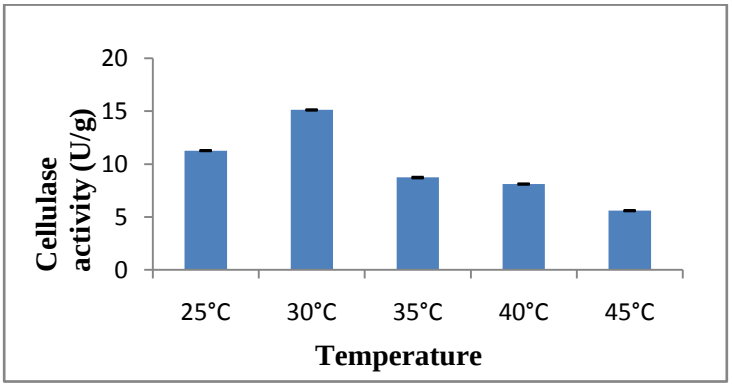

Figure 3: Impact of temperature on cellulase activity (U/g) using 5% substrate concentration.

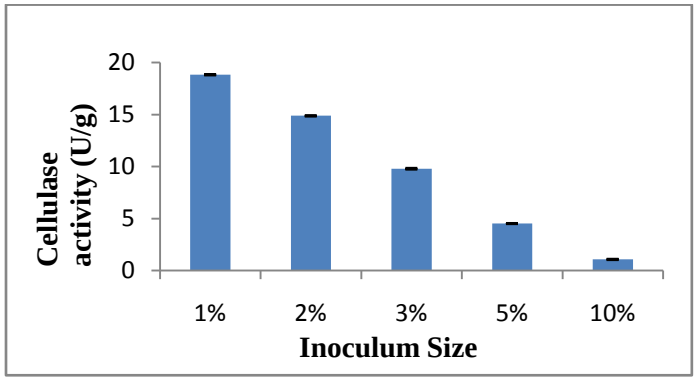

Figure 4: Impact of Inoculum size on cellulase activity (U/g) at pH7.0±0.2; 30 ^{0}C and 5% substrate concentration.

C.Optimization of temperature:

The observations were carried out by *P. aeruginosa*MTCC 4643 on cellulase activity at various temperatures ranging from 25-45^{0}C at 5% concentration of saw dust with pH of 7.2± 0.2 as shown in Figure 3. Temperature strongly affects the conversion of lignocellulosic substrate into end product, which varies according to the organism involved even slight changes in temperature can affect cellulase production. (Vu*et al.*2011).The optimum cellulase activity (15.12 U/g) was observed at 30^{0}C**.**Bakare *et al.* (2005) recorded the optimum temperature of 35^{0}C for best production of enzyme by *Pseudomonas fluorescens*using CMC as a substrate.Rajoka(2004) showed that an optimum temperature for *Cellulomonas flavigena*was 30^{0}C when cellulose and sugar cane bagasses used as a substrate respectively.

D.Optimization of inoculum size:

The initial inoculum level in the media is a critical factor in fermentation process **(**Shankar and Isaiarasu, 2011).The effect of various inoculum size of 1-10% was tested as presented inFigure4. The maximum cellulase activity (18.83 U/g) was found at 1% v/v using 5% of saw dust with temperature of 30^{0}C and pH of 7.2± 0.2. Decline productions were observed by increasing the inoculum's concentration of 2% v/v.Shankar and Isaiarasu (2011) found 2% inoculum size optimum for the cellulase activity when CMC used as a substrate by*Bacillus pumilus*.

IV. CONCLUSION

In conclusion, *Pseudomonas aeruginosa* MTCC 4643 is capable of producingcellulases from sawdust. Sawdust is low-cost lignocellulosic waste materialand potentially useful forcommercial cellulase production.

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