ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 7, pp. 18139-18142, July, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

PRODUCTION OF CELLULASE FROM COCONUT COIR WASTE BY *BACILLUS SUBTILIS* UNDER SOLID STATE FERMENTATION

Bhagyashri Poddar., Rutuga Manthanvar and Anita Chandak*

Department of Microbiology, Kamla Nehru College, Nagpur

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0807.0458

ARTICLE INFO

Article History:

Received 15th April, 2017
Received in revised form 25th May, 2017
Accepted 23rd June, 2017
Published online 28th July, 2017

Key Words:

Cellulase, *Bacillus subtilis*, coconut coir waste, solid state fermentation.

ABSTRACT

The aim of present work was focused on the cellulase production by *Bacillus subtilis* using coconut coir waste as a substrate. Coconut coir waste is an abundant natural biopolymer on earth and most dominating Agricultural waste. These agro industrial residues are cheap raw material for cellulase production. The maximum production of cellulase (in terms of glucose production) was obtained after 7 days of incubation period in solid state fermentation. The physical and nutritional parameters like pH, temperature, moisture content, and incubation period and nitrogen sources are optimized. The optimal condition for maximum biosynthesis of cellulose by *B. subtitles* were shown to be at pH 7.0, temperature 40^0C, moisture ratio 1:1.5 (w/v), incubation period 7 days and in $(NH_4)_2SO_4$ as nitrogen source.

Copyright © Bhagyashri Poddar *et al*, 2017, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Coconuts are produced in 92 countries worldwide on about more than 10 million hectares. Indonesia, Philippines and India account for almost 75% of world coconut production with Indonesia being the world's largest coconut producer. A coconut plantation is analogous to energy crop plantations; however coconut plantations are a source of wide variety of products in addition to energy. The coconut fruit yields 40% coconut husks containing 30% fibre, with dust making up the rest. The chemical composition of coconut (cocoas nucifera) husk, consist of 39.31% Alpha cellulose, 16.15% hemicelluloses, 29.79% lignin and 38.48% extractives.

Cellulases break down the cellulose molecule into monosaccharide's ("simple sugars") such as beta-glucose, or shorter polysaccharides and oligosaccharides. Cellulose breakdown is of considerable economic importance, because it makes a major constituent of plants available for consumption and use in chemical reactions. The specific reaction involved is the hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose, hemicelluloses, legnin, and cereal beta-D-glucans. Because cellulose molecules bind strongly to each other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides such as starch.

Cellulase is any of several enzymes produced chiefly by fungi, bacteria, and protozoan's that catalyze cellulolysis, the decomposition of cellulose and of some related polysaccharides. Most mammals have only very limited ability to digest dietary fibres such as cellulose by themselves. In many herbivorous animals such as ruminants like cattle and sheep and hindgut fomenters like horses, celluloses are produced by symbiotic bacteria. Celluloses are produced by a few types of animals, such as some termites.

Cellulases contribute to 8% of the worldwide industrial enzyme demands. The major industrial application 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. Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juice and in baking, while de-inking of paper is yet another emerging application. A potential challenging area where celluloses would have a central role is the bioconversion of renewable cellulosic biomass to commodity chemicals. Application of this enzyme in detergent, leather and paper industries demands identification of highly stable enzymes active at extreme pH and temperature.

Since the production of cellulose enzyme is a major factor in hydrolysis of cellulosic material, it is important to make the process economically viable; this study therefore investigated on the bioconversion of coconut coir waste (which could cause

Corresponding author:* **Anita Chandak
Department of Microbiology, Kamla Nehru College, Nagpur

pollution to the environment) into a more useful product (cellulose) using *Bacillus subtilis*.

MATERIALS AND METHODS

Isolation and screening of Bacillus subtilis

Cellulase producing bacteria were isolated from soil by the dilution pour plate or spread plate method using carboxymethyl cellulose (CMC) agar media. The plates were incubated at 40^0 for 24 hours. To visualize the hydrolysis zone the plates were flooded with an aqueous solution of 0.1 % Congo-red for 15 min and washed with 1M Nacl. To indicate the cellulose activity of the organisms, diameter of the clear zone around colonies on CMC agar was measured.

Preparation of inoculums

The isolated colonies of *Bacillus subtilis* on CMC agar plate were individually maintain on carboxymethyl cellulose (CMC) agar slant at 40^0C for 48 hours. It was then subculture on fresh sterile inoculums media comprised of ($MgSO4.H_2O$ – 0.03g, K_2HPO_4 0.2 g, glucose-g, $(NH_4)_2SO_4$ – 0.05 g , peptone - 1g at pH-7.0 into 100mL of distilled water) and incubated and maintain at 40^0 C for 24 hours and used throughout the experiment.

Pre-treatment of coconut waste

The substrate used for this work was coconut coir waste. Coconut coir waste were first ground to fine powder and then it was individually treated with 1 % (w/v) Noah solution in the ratio of 1:10 (substrate: solution) for 1 hour. This was then brought to neutral pH by washing thoroughly with distilled water. The pre-treated coconut coir waste were dried at room temperature and further made on to powder form in an electric blender.

Solid state fermentation for cellulase production

Solid state fermentation was carried out in 250ml Erlenmeyer flasks that contained 10g of coconut coir waste and 15 ml of basal medium. The basal medium comprised of (K_2HPO_4 – 0.1 g ; $MgSO_4$ - 0.003 g ; $(NH_4)_2SO_4$ 0.2g ; $MnCl_2.4H_2O$ – 0.05g; $FeSO_4.7H_2O$ - 0.025 g yeast extract - 0.5g ; Distilled Water 100 mL at pH 7.0) . The flask was sterilized at 121^0C for 15 min and Cooled to room temperature. About 1mL of inoculums was added. Mixed well and incubated at 40^0C in a humidified incubator for 7 days. The flasks were periodically mixed by gentle shaking.

Enzyme extraction

22ml of 0.1M phosphate buffer saline (pH 7) was added to each of the inoculated substrate beds after 7 days of incubation periods and was vigorously shaken in rotary shaker for 15 min at 120rpm. The mixture was filtered through cheese cloth and centrifuged at 6000 rpm for 15 min. The supernatant was filtered through cheese cloth and the filtrate was used as the crude enzyme preparation

Cellulase Activity

1.8 ml of 1 % carboxymethyl cellulose (CMC) in 0.1M citrate buffer pH 5.6 was placed in a test tube and 0.2 ml of culture filtrate was added. The test tube was incubated at 40^0C in a water bath with shaker for 30 min. The reaction was terminated by adding 3ml of 3, 5-dinitrosalicylic acid (DNS) reagent to the reaction mixture heated in boiling water bath for 5 min. The absorbance of the approximately diluted reaction mixture was read at 540nm using a calorimeter.

Optimization of process parameter

Effect of pH: Solid state fermentation investigated the effect of pH on cellulose enzyme production by adjusting pH of basal salt solution to 6.0, 7.0, 8.0, 9.0, 10.0. The substrate were then incubated for 4 days at 40°C in incubator

Effect of Temperature: The effect of temperature on cellulose enzyme production was investigated by solid state fermentation in coconut coir substrate and incubated at 30°C, 40°C, 50°C, 60°C at pH 7.0 for 4 days

Effect of Incubation period: The effect of incubation period on cellulose enzyme production was investigated by checking the enzyme activity from 1-9 days of incubation at pH 7.0 at 40°C.

Effect of Moisture content: Solid state fermentation investigated the effect of moisture content on cellulose enzyme production by varying the volume of basal salt solution to 10mL, 15mL, 20mL, and 25mL. The substrate was then incubated for 4 days at 40^0C in incubator.

Effect of Nitrogen sources: The effect of nitrogen sources on cellulose enzyme production was studied by replacing the nitrogen source $(NH_4)_2SO_4$ in basal salt solution at pH 7.0 with 0.2g of $NaNO_3$, NH_4Cl, NH_4NO_3, KNO_3 and incubated for 4 days at 40°C.

RESULTS

Solid state fermentation

The enzyme cellulose was produced by *Bacillus subtilis* using coconut coir waste as a substrate. The media and culture conditions like substrate (coconut coir waste), pH, temperature, incubation period, moisture content and nitrogen source were further optimized for the maximum production of cellulose.

For the production of cellulose the media was incubated for 7 days and after incubation period O.D. was recorded. The O.D. was plotted on the standard glucose graph and glucose concentration was calculated. The glucose concentration was found to be between 485-500ug/ml. (fig: 1)

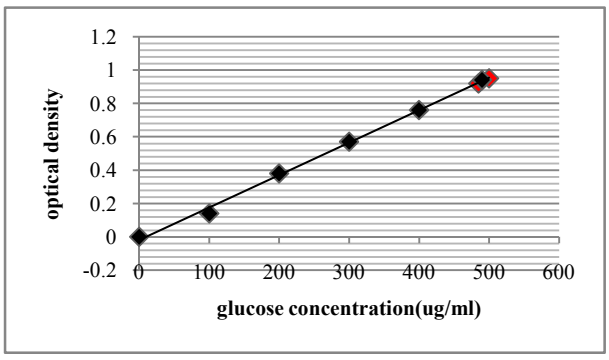

Fig 1 Standard Glucose Concentration Curve

Concentration of standard glucose
Concentration of glucose present in coconut coir waste

Effect of pH

Bacillus subtilis were allowed to grow in a media at different pH ranging from 6.0 - 10.0. The pH change observed during the growth of microbes affect the product stability in the medium. Optimum pH for maximum production of cellulose (in terms of glucose concentration) was found to be 7.0. (fig: 2)

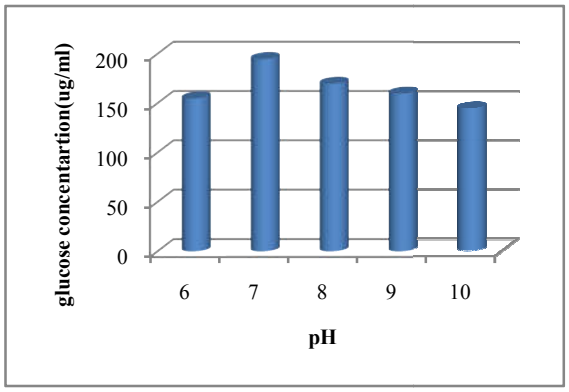

Fig 2 Effect of pH on cellulase production

Effect of temperature

Incubation temperature plays an important role in the metabolic activities of micro-organisms. In the present study enzyme activity recorded at different temperature revealed that *Bacillus subtilis* show maximum glucose concentration on coconut coir waste at 40^0 C. (fig: 3)

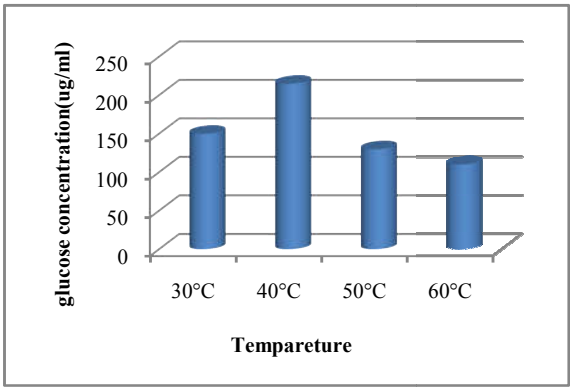

Fig 3 Effect of temperature on cellulose production.

Effect of incubation period

After incubation period it was found that *Bacillus subtilis* shows lower level of glucose concentration from 1-4 day. Glucose concentration increases from 5-7 day.

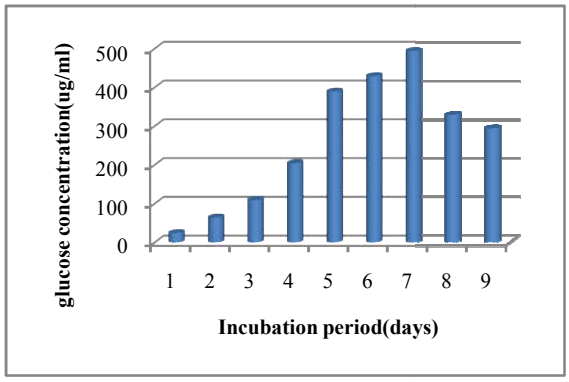

Fig 4 Effect of Incubation period on cellulose Production

The glucose concentration was significantly reduced after 7 days due to depletion in nutrients or accumulation of other by-products in fermentation or basal media which leads to decrease in glucose concentration. (Fig: 4)

Effect of moisture content

Moisture content is a critical factor in solid state fermentation because this variable has influence on growth, biosynthesis and secretion of enzyme. In the present study *Bacillus subtilis* allowed to grow in different volume of fermentation media ranging from 10ml to 25ml. Maximum glucose concentration was observed when volume of media was maintained 15ml. Any further decrease or increase in moisture ratio decreases the glucose concentration. (Fig: 5)

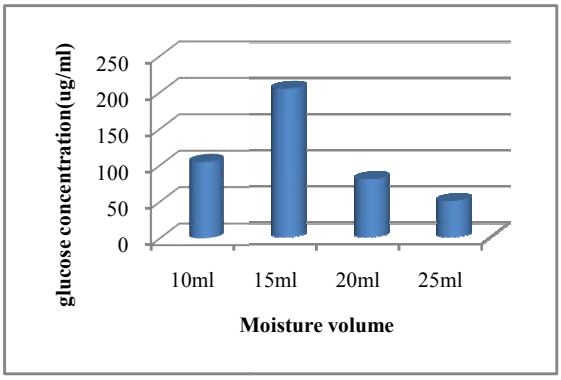

Fig 5 Effect of moisture content on cellulase production.

Effect of nitrogen source

The effect of nitrogen sources was studied in the growth medium, where Ammonium sulphate $[(NH_4)_2SO_4]$ was replaced by sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl), ammonium nitrate (NH_4NO_3) AND potassium nitrate (KNO_3). Among all the nitrogen sources, it was found that ammonium sulphate $[(NH_4)_2SO_4]$ was best nitrogen source for cellulose production. (Fig: 6)

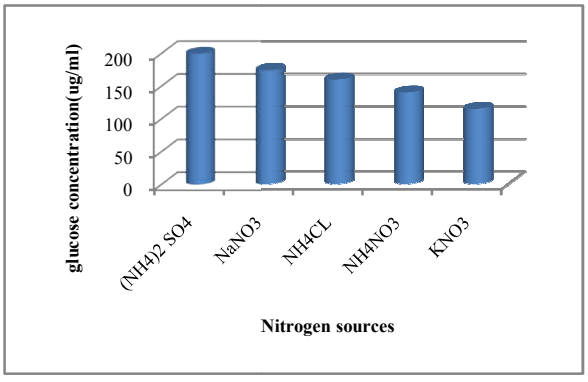

Fig 6 Effect of nitrogen sources on cellulose Production

DISCUSSION

Enzymes are large biological molecules responsible for the thousands of metabolic processes that sustain life. They are important product obtain for human needs from several sources are generally used in industries.

Enzymes remain a major cost factor for the nascent lignocelluloses fuels and chemical industries. In recent study most of the enzymes are produced from lignocelluloses material by fermentation process. An important consideration

in fermentation process is the cost of the carbon source, which contributes significantly to overall production expenses. Accordingly, coconut coir waste which contains amorphous cellulose, crystalline cellulose and hemicelluloses was examined as substrate to cellulose production by *Bacillus subtilis.*

Bacillus subtilis grown rapidly on coconut waste utilizing it as a source of carbon and energy and give promising level of cellulose enzyme (glucose concentration) in 7 days of incubation period. Maximum cellulose production was found until 7 days and after 7 days where culture reaches the death phase cellulose production goes on decreasing.

Restage et. Al reported that maximum production of cellulose by cellulose degrading Bactria on day 10, when the culture reaches the death phase, while maximum cellulase activity was observed on day 7, at the end of exponential growth phase, after which cellulase production decreases with time.

Yogita Lugani, Rajesh singla and Balwinder Singh reported that when *Bacillus sp. Y3* was allow to grow on the CMC the maximum cellulase production was observed at 96 hours of incubation period. The cellulase production was significantly reduced after 96 hours due to depletion of nutrient or accumulation of other by-product in the fermentation media which leads to decrease in cellulase production.

Das et. Al also reported the the maximum cellulase production was obtained after 96 hours by thermopile *Bacillus* sp.

PH variation, temperature variation, moisture content and effect of different nitrogen sources on production of cellulase was also evaluated during cultivation of *Bacillus subtitles.* The cellulase production was maximum when culture was allowed to grow in optimized condition. The optimum conditions such as pH (7.0), temperature (40^0C), moisture content (15ml), nitrogen source (ammonium sulphate $[(NH_4)SO_4]$ was found to be responsible for maximum production of cellulase from coconut waste. Any further variation in pH, temperature, and moisture content and nitrogen source decreases the production of cellulase by *Bacillus subtilis*.

Deepmoni Deka, Saprativ P. Das, Naresh Sahoo, Debasish Das, Mohammad Jawed, Dinesh Goyal and Arun Goyal reported that when the culture level of 40^0C an enhancement in cellulase production was achieved. At the optimized physical parameter of pH 7.2, temperature 40^0C and agitation speed 121rpm, the fermentation by *Bacillus subtilis* showed 33% enhancement in cellulase production as compared to unoptimized parameters.

Sonia Sethi, Aparna Datta, B. Lal Gupta and Saksham Gupta reported that the *Bacillus subtilis* gives maximum cellulase production at optimized physical parameter pH 7, temperature 40^0C, carbon source fructose, and ammonium sulphate as nitrogen source when allow to grow on coconut- waste.

References

Alam, M. Z., 2004. Study of five cellulolytic microorganisms and their celluloses. MSc Thesis, Chittagong University Bangladesh. 1-132

B.V.KiliKian, L.C Afonso and I.R. Pinheiro, 2014. Filamentous fungi and media for cellulose production in solid state cultures. *Braz. J microbial*. 11; 45(1):279-286

Bajpai, P., 1999. Applications of enzymes in the pulp and paper industry. *Biotechnology progress*. 15:147-157.

Bhat, M. K. and S. Bhat, 1997. Cellulose degrading enzymes and their potential industrial applications. *Biotechnol Adv*., 15: 583-620.

Jwan J.Abdullah, Darren Greethan, Nanttha Pensupa, Gregory A.Tucker and Chenyu Du., 2016.Optimization of cellulose Production from Municipal solid waste (MSW) Using solid state fermentation. *J. Fundam Renewable Energy* Appl. 6:206.

Muhammad Imran, Zahid Anwar, Muhammad Irshad, Muhammad Javaid asad, Hassan ashfaq, 2016. Cellulase Production from species of fungi and bacteria from agricultural wastes and its utilization in industry. *Advances in enzyme research* vol. 04 no. 02

P.Sarvanan, R. Muthuvelayudham, T.Viruthagiri, 2013. Enhanced production of cellulose from pineapple waste by Response surface Methodology. *Journal of engineering* vol 2013, Article ID 979547, 8 pages.

P.Shobana, N.Uma Maheswari, 2013. Production of cellulose from *Aspergillusfmigatus* under Submerged and solid state fermentation using Agricultural waste. *IJAPBC* VOL.2(4)

Rajeev Gaur and soni Tiwari, 2015. Isolation, production, purification and characterization of organic-solvent-thermosatble alkalophilic cellulose from *Bacillus Vallismortis* RG-07. *BMC Biotechnology*. 15:19

Result *et al*., 2015. Screening and characterization of cellulose producing bacteria from soil and molasses of sugar industry. *IJB*, vol. 6, no.3, p.230-238.

Sangria Sadhu and Tushar kanti Maiti, 2013. Cellulase production by Bacteria. *British microbiology research journal*. 3(3):235-258

Saraswati Bai, M. Ravi kumar. D.J Mukesh Kumar, P.Balashanmugam, M.D Bala Kumaran P.T. Kalaichelvan, 2012. Cellulase production by *Bacillussubtilis* isolated from cow dung. *Achieves of Applied science research.* 4(1):269-279

Silvania A. Ladeira, Erica Cruz, Meire Lelis Leal Martine, 2015. Cellulase production by thermopile *Bacillus* sp. SMIA-2 and its detergent compatibility. *Electronic Journal of Biotechnology* vol. 18(2):110-115

Soma Mrudula, Rangasamy Murugommal, 2011.Production of cellulose by *Aspergillusniger* under submerged and solid state fermentation using coir waste as a substrate. *Braz. J microbial*. vol. 42 no. 3

Vipul Verma, Alpika Verma and Akhilesh Kushwa, 2012.Isolation and production of cellulose enzyme from bacteria isolated from agricultural fields. *Advances in applied sci research*. 3(1):171-174

Wiping yang, Fanxu Meng, Binyun Cao, 2014. Isolation and identification of a celluloytic bacterium from the Tibetan Pig's intestine and investigation of its cellulose production. *Electronic journal of Biotechnology* vol. 17(6):262-267

Yogita Lugani, Rajesh Singla and Balwinder Singh soodh, 2015. Optimization of cellulose production from Newly Isolated Bacillus sp.y3. *J. Bioprocess Biotech* 5:264