Bioprocessing of Agricultural Waste (Banana Pseudostem) by Solid-State Fermentation (SSF) for Production of Cellulase

Sharanappa Achappa a*, Anil R. Shet a, Veeranna S. Hombalimath a, Laxmikant R. Patil a and Shivalingasarj V. Desai a

a Department of Biotechnology, KLE Technological University, Hubballi-580031, India.

ABSTRACT

Cellulase was produced by Cellulomonas uda utilizing banana stem waste by solid state fermentation (SSF). The different parameters effects considered like particle size of substrate, pH (incubation and extraction), incubation period, temperature (incubation and extraction), media components (yeast extract and peptone) and moisture of substrate on the production of cellulase was investigated. The optimum activity of cellulase produced by Cellulomonas uda on banana waste for particle size (6.97 IU/min for 1mm), extraction pH (7.13 IU/min for pH 7), incubation pH (6.97 IU/min for pH 7), extraction temperature (7.10 IU/min for 500C), incubation temperature (7.20 IU/min for 450C), incubation period (7.20 IU/min on 3rd day), moisture content (7.12 IU/min for 100% ), peptone content (7.23 IU/min for 0.5 gm) and yeast extract content (7.18 IU/min for 0.30gm) was recorded. The cellulase produced by Cellulomonas uda by using banana stem waste indicates the socio-economic utilization of agricultural waste. After purification and characterization; cellulase enzyme can be used in industrial processes.

Keywords: Banana waste; cellulose; cellulomonas uda; solid state fermentation.

*Corresponding author: E-mail: sharanappaa@kletech.ac.in;
1. INTRODUCTION

Cellulose represents about $1.5 \times 10^{12}$ tons of biomass produced by photosynthesis and is most inexhaustible raw material source for different products [1]. Cellulose is the most dominating agricultural waste and is considered as abundant and renewable biopolymer present on planet earth [2]. Hydrolysis of lignocellulosic waste by microbial degradation and utilization of resultant sugars (reducing) by fermentation process for production of bioproducts or biofuel will be most promising strategy for efficient utilization of this biomass [3]. Different class of Cellulases enzyme produced by a number of microorganisms helps in degradation of lignocellulosic waste through fermentation process [4]. Media used in cellulose fermentation consists of cellulose in different degree of purity for submerged fermentation [5] and raw lignocellulosic substrate for solid state fermentation [6].

Cellulases hydrolyze cellulose ($\beta$-1,4-D-glucan linkages) and produce as primary products glucose, cellobiose and cellobio-oligosaccharides. There are three major types of cellulase enzymes [Celllobiohydrolase (CBH or 1, 4- $\beta$-D-glucan cellobiohydrolase, EC 3.2.1.91), Endo-$\beta$-1,4-glucanase (EG or endo-$\beta$-1,4-$\beta$-D-glucan 4- glucanohydrolase, EC 3.2.14) and $\beta$-glucosidase (BG-EC 3.2.1.21)] [7].

Cellulase is considered as the third largest group of extracellular enzyme used in different industrial applications [8] other then lipase [9,10], xylanase [11,12], amylase [13], protease[14] and pectinase [15].

Cellulases enzymes used in different industries like textile industry [16], manufacturing of detergents [17,18], paper and pulp industry [19], animal feeds [20] and food industry [21]. In beverage industry, cellulases enzyme is used in clarification and extraction of vegetable and fruit juices, fruit purees and nectars production, and in olive oil extraction [21]. Glucanases enzyme are added in beer manufacturing to enhance the process of barley malting [22], exogenous hemicellulases and glucanases used in wine industry for color extraction and better maceration [21]. Cellulases and hemicellulases have been used for hand sheet strength properties and biomechanical pulp in the paper and pulp industry [23], deinking of recycled fibers [24]. Extracellular enzymes are also used in synthesis of silver nanoparticles [25,26], dye degradation [27], immobilization [28,29] and bio-diesel production [30,28].

A most prospective use of cellulase is the conversion of cellulosic or lignocellulosic raw materials to other fermentable sugars and glucose, which acts as a microbial carbon source for different bioproduct production like single cell proteins and ethanol [31,32]. Commercial production of enzyme cellulases can be achieved by either solid [3] or submerged fermentation process [1] culture including batch, fed batch, and continuous fermentation processes [3]. Various statistical tools or methods are used in order to optimize production of cellulase or biomolecules by using fermentation process [33,34,35].

The main agricultural waste of Jalgaon district is the banana fruit stalk and pseudo stem, also accumulated as waste, posing serious environmental problems. Banana fruit stalk contains total sugar (56.8%), starch (27.0%), reducing sugar (4.65%) and protein (4.3%) on a dry weight basis. The Banana pseudo stem consists of Hemicellulose 65%, Cellulose 64%, Lignin 19%, and Ash 15% on dry weight basis. The main objective of our research work was to effectively utilize the agricultural waste (banana pseudo-stem) for the production of cellulase from microbial strain by SSF. One-Factor at a Time (OFAT) study was conducted to select the optimize operating parameters and media components for the production of microbial cellulase.

2. MATERIALS AND METHODS

2.1 Substrate Collection

The main agricultural waste of Jalgaon District (called land of Banana) is banana pseudostem. Pseudostem rich in cellulose accumulate as waste in the banana field. This pseudostem collected from the banana fields around Jalgaon city is utilized as substrate in SSF. Banana pseudostem collected from the fields near North Maharashatra University, Jalgaon.

2.2 Microorganism

Microorganism was procured from National Center for Industrial Microorganism (NCIM), National Chemical Laboratory (NCL), Pune. *Cellulomonas uda* NCIM No. 2353 (Cellulase producer) is maintained on nutrient agar slant (Peptone 5 gm, Beef extract 3 gm, NaCl 5 gm, Distill water 1000 ml) of pH 7 at 4°C. The
subculturing of microorganism was done regularly in the department laboratory.

2.3 Preparation of Substrate

Banana stem waste was considered as substrate for cellulase enzyme production was obtained from farm land located back side of North Maharashtra University, Jalgaon. The collected substrate was chopped in to smaller pieces and for 48hrs kept under sun light for sun drying. After sun drying, substrate was dried at 70°C for 24 hrs in hot air oven. The substrate was powdered by using electrical grinder.

The powdered substrates were passed through sieve shaker of 2mm mesh size. Substrate after sieve analysis were collected in conical flask of 250ml or petriplates. Salt solution was used to moisture the substrate with a composition of gm / 100 ml: peptone 0.5, yeast extract 0.3, Na₂HPO₄.2H₂O 1.1, NaCl 1.5, KCl 0.3, MgSO₄.7H₂O 0.01, and NaH₂PO₄ 0.61 in laminar air flow [3,13]. The substrates was moistened by salt solution upto 150% (W/V). Moistened substrate was autoclaved(sterilization process) at 121°C for 15 minute to increase its amenability for microorganisms.

2.4 Preparation of Inoculum

Cellulomonas uda (NCIM No. 2353, Cellulase producer) cells were aseptically transferred to conical flask of 100 ml containing 50 ml of sterilized medium (sterilized for 15 minutes at 121°C) containing g/100ml: glucose 2, peptone 0.5, yeast extract 0.3, Na₂HPO₄.2H₂O 1.1, NaCl 1.5, KCl 0.3, MgSO₄.7H₂O 0.01 and NaH₂PO₄ 0.61. The conical flask was incubated in bacteriological incubator for 48 hrs at 37°C. The microbial suspension (10⁶ – 10⁷ cells / ml) of homogeneous form was used as inoculum.

2.5 Solid State Fermentation

Sterilized substrate was cooled to required temperature. Substrate of weighing 10gm in petridish and 15gm in 250ml was taken. Later inoculum of measuring 10 % (W/V) was added in the laminar air flow by using sterilized pipette.

Cellulomonas uda (NCIM No. 2353, Cellulase producer) was inoculated on banana stem. After inoculation, petriplates and conical flask was incubated at 37°C for 3days. Conical flasks were gently shaken for every 12 hrs for mixing of solid substrate with microorganism.

2.6 Extraction of Cellulase Enzyme

After fermentation process, 1:10 (W/V) of 0.1 M sodium phosphate buffer of pH 6.9 was mixed with fermented banana stem waste for extraction of crude cellulase enzyme. The mixture was kept at rotary flask shaker at 150 rpm for 60 minutes. The mixture was filtered by using muslin cloth. Collected filtrate was centrifuged for 10 minutes at 10000rpm at 4°C. Collected supernatant was used as crude cellulase extract for estimating cellulase activity [13].

2.7 Cellulase Enzyme Assay

Activity of cellulase enzyme was estimated by filter paper method. Cellulase enzyme acts on cellulose present in filter paper to produce reducing sugar (glucose) and measured by DNSA method. Chemicals used for enzyme estimation were sodium phosphate buffer (pH 6.9) of 0.1 M, filter paper discs, DNSA, potassium sodium tartarate, glucose solution. 1 ml of crude enzyme extract was added to 30 mg of dry whatmann filter paper 1 and incubated the mixture for 1 hr at 50°C. Added 2.5ml of DNSA reagent and mixture was heated to 15min in a boiling water bath. Absorbance was measured at 530 nm. Standard graph of glucose was prepared. The enzyme activity was expressed as μmol of glucose released per minute.

2.8 Parameters Considered for SSF

The effect different parameters like particle size of substrate, pH (incubation and extraction), incubation period, temperature (incubation and extraction), media components (yeast extract and peptone) and moisture content on cellulase production was investigated.

3. RESULTS AND DISCUSSION

3.1 Effect of Substrate Particle Size on Cellulase Activity

Effect of varying particle size on cellulase enzyme activity was considered by taking banana stem as solid substrate. Substrate from 0.100 to 2mm particle size was considered to its effect of different particle size on cellulase activity. Grinded substrate was sieved to different particle size by using sieve shaker. Sieves of different mesh size arranged in a decreasing order of mesh size as 2 mm, 1.4 mm, 1 mm, 0.850 mm, 0.425 mm, 212mm, 106 mm were
arranged on a vibrator. Substrate collected on the sieve for example 1mm by passing through sieve of 1.4mm was considered as 1.4mm particle size. Different particle sized substrate was taken in 250 ml conical flask and SSF was performed for 36 hrs. at 37 °C. Crude enzyme was extracted from each conical flask containing different particle sized substrate and cellulase activity was recorded. Fig. 1 shows the mean reading of cellulase activity against particle size. Substrate with larger particles have larger interparticle space thereby providing better respiration/aeration efficiency. In contrast, substrate of smaller particle size results in accumulation of substrate, thereby interfere with microbial aeration/respiration by resulting in poor growth and cellulase enzyme production. For cellulase activity, increase in cellulase activity was resulted with decrease in particle size from 2mm to 1mm, but further decrease in particle size of substrate up to 0.106mm decreases cellulase activity. Optimal activity of 6.97IU/min was observed at 1mm particle size.

To investigate the effect different parameters like pH (incubation and extraction), incubation period, temperature (incubation and extraction), media components (yeast extract and peptone) and moisture content on the production of cellulase, 1mm substrate particle size was used for the SSF.

### 3.2 Effect of Extraction pH on Cellulase Activity

Extraction pH and its effects on cellulase activity was performed by SSF. Cellulase was extracted by buffers of pH from 4 to 9. For pH 4 and 5, 0.2M Acetate buffer was used. For pH 6, 7, and 8 Phosphate buffer of 0.1M was used. For pH 9, 0.2M Glycine buffer was used. To study the effect of extracting pH and also to optimize the condition for pH, the cellulase enzyme activity was recorded. Change in the concentration of hydrogen ion will have considerable influences on enzyme activity.

Each enzyme will have its maximum activity at its optimum pH. Amino acids ionic charges particularly at the substrate binding site and active site, etc is influenced by presence of hydrogen ions which in turn influence the enzyme activity. For cellulase activity, increase in enzyme activity was observed from pH 4 to 7 increases, further increase in pH up to 9, decreases enzyme activity. Optimal cellulase activity of 7.13IU/min was observed at pH 7 as shown in Fig. 2. Muhammad Irfan et al. 2010 reported production of carboxymethyl cellulase by thermophilic *Trichoderma viride* on wheat straw and got optimum activity for extraction pH at pH 5.5 [36].

![Fig. 1. Effect of substrate particle size on cellulase activity](image-url)
3.3 Effect of Incubation pH on Cellulase Activity

Effect of pH (incubation) on cellulase enzyme activity was performed by SSF. Among different physicochemical parameters, growth medium pH have a major role in inducing enzyme secretion and changes in morphological structure in the organism. Medium pH varies due to consumption of substrate (eg: protein hydrolysis) and production of metabolites like organic acids. Cellulase activity increases with, increase in pH from 4 to 7, further increase in pH up to 9 decreases cellulase activity. At pH 7 optimal cellulase activity of 6.97 IU/min was observed. Muhammad Irfan et al. 2010 reported production of carboxymethyl cellulase by thermophilic Trichoderma viride FBL1 on wheat straw and got optimum activity at pH 5 [36]. S. Shafique et al. 2004 reported production of exoglucanase by Bacillus subtilis on banana stalk and got optimum activity at pH 5 [36]. S. Shafique et al. 2004 reported production of exoglucanase by Bacillus subtilis on banana stalk and got optimum activity at pH 7 [36]. Ikram Ul Haq et al. 2006 reported production of cellulase by Trichoderma harzianum on agricultural by products and got optimum activity at pH 6.5 [38]. Ishtiaq Ahmed et al. reported production of cellulase by Trichoderma viride on wheat straw and got optimum activity at pH 5.5 [39].

Fig. 2. Effect of incubation pH on cellulase activity

Fig. 3. Effect of incubation pH on cellulase activity
3.4 Effect of Incubation Temperature on Cellulase Activity

Solid state fermentation was performed to study the effect of incubation temperature on cellulase enzyme activity. Incubation temperatures used were 20°C, 25°C, 30°C, 35°C, 40°C, 45°C and 50°C. SSF was performed by incubating conical flask at different temperature for 3 days. Crude cellulase enzyme was extracted and activity was measured as shown in Fig. 4. Incubation temperature induces changes in cell wall and cell membrane thereby effecting the control of enzyme secretion to cultural media. Cellulase activity increased with increase in temperature from 25°C to 45°C, whereas increase in temperature to 50°C decreased the enzyme activity. At 45°C of incubation temperature, optimum cellulase activity of 7.20IU/min was observed. Muhammad Irfan et al. 2010 reported production of carboxymethyl cellulase by thermophilic Trichoderma viride on wheat straw and got optimum activity at 40°C [36]. Shafique et al. 2004 reported production of exoglucanase by Bacillus subtilis on banana stalk and got optimum activity at 40°C [37]. Ikram Ul Haq et al. 2006 reported production of cellulase by Trichoderma harzianum on agricultural by products and got optimum activity at 28°C [39]. Ishtiaq Ahmed et al. reported production of cellulase by Trichoderma viride on wheat straw and got optimum activity at 40°C [40].

3.5 Effect of Extraction Temperature on Cellulase Activity

SSF was performed to study the effect of extraction temperature on cellulase activity. Temperatures considered were 25°C, 30°C, 35°C, 40°C, 45°C and 50°C. After SSF, crude cellulase was extracted and activity was checked at different temperature. Increase in temperature increases enzyme activity to a maximum and then declines. Increase in temperature to its optimum value increases the molecular activation energy, thereby increases molecular collision resulting in increase in rate of reaction. Above optimum temperature, enzyme denaturation takes place resulting in dearrangement in native structure of the protein/enzymes, which results in deactivation of enzymes. For cellulose activity, increase in temperature from 25°C to 50°C increases enzyme activity. The optimum enzyme activity obtained was 7.10 IU/min at 50°C as shown in Fig. 5. Muhammad Irfan et al. 2010 reported production of carboxymethyl cellulase by thermophilic Trichoderma viride on wheat straw and got optimum activity at 55°C [36].

![Fig. 4. Effect of incubation temperature on cellulase activity](image-url)
Fig. 5. Effect on cellulase activity by extraction temperature

3.6 Effect of Incubation Period on Cellulase Activity

SSF was performed by altering incubation time/period from 1 day to 10 days at 45°C. For cellulase activity, incubation time/period from day 1 to day 3, increases cellulase activity. Further incubation time/period from 3rd day to 10th day, cellulase activity was decreased. At 3rd day of incubation period, optimum cellulase activity of 7.20IU/min was observed. This may be due to availability of required moisture content in the solid substrate, whereas drastic decrease in cellulase activity was observed after 3rd day of incubation due to decrease in moisture content of the substrate. Muhammad Irfan et al. 2010 reported production of carboxymethyl cellulase by thermophilic Trichoderma viride on wheat straw and got optimum activity for incubation period at 7th day incubation [36]. MAM Abo-State et al 2010 reported production of cellulase by Aspergillus terreus mam-F23 and Aspergillus flavus mam-F35 on wheat straw and got optimum activity for incubation period at 48 hrs and 60 hrs respectively [41]. S. Shafique et al 2004 reported production of exoglucanase by Bacillus subtilis on banana stalk and got optimum activity at 72 hrs of incubation [37]. Ezyana Kamal Bahrin et al. 2011 reported production of cellulase by Botryosphaeria sp. from oil palm empty fruit bunch and got optimum activity at 3rd day of incubation [42]. C. Pothiraj et al. 2006 reported production of cellulases by various fungal cultures like Rhizopus stolonifer, Aspergillus niger and Aspergillus terreus on cassava waste and got optimum activity at 10 days, 8 days and 8 days of incubation period respectively [43]. Ikram Ul Haq et al. 2006 reported production of cellulase by Trichodrma harzianum on agricultural by products and got optimum activity at 72 hrs of incubation [38].

3.7 Effect of Substrate Moisture Content on Cellulase Activity

SSF was performed to study the effect of substrate moisture content on cellulase activity. Different moisture content of substrate was achieved by adding salt solution of w/v from 50 to 120%. In SSF, growth of microorganism and enzyme secretion is influenced by initial moisture contents of the substrate. Presence of moisture in the substrate helps microorganism to access nutrients present in substrate thereby increases microorganism growth. Presence of moisture in substrate influences the physicochemical properties of the substrate, thereby effect the production of enzyme. Higher moisture content of the substrate results in substrate particles to stick together and thereby affecting the diffusion of oxygen between the substrate pores. This in turn reduces the growth of microorganism due to steric hindrance caused due to reduced porosity of the substrate and thus decrease in enzyme production.

Lower substrate moisture content decreases the solubility of nutrients of the substrate, low degree of swelling and high water tension. For cellulase activity, moisture content from 50% to 100% increases cellulase activity, further change in...
moisture content of substrate from 100% to 120% decreases cellulase activity. At 100% moisture content, optimum cellulase activity of 7.12IU/min was observed as shown in Fig. 7. Muhammad Irfan et al. 2010 reported production of carboxymethyl cellulase by thermophilic *Trichoderma viride* on wheat straw and got optimum activity for initial moisture content at 40% [36]. S. Shafique et al. 2004 reported production of exoglucanase by *Bacillus subtilis* on banana stalk and got optimum activity at 70% of initial moisture content [37]. Ishtiaq Ahmed et al. reported production of cellulase by *Trichoderma viride* on wheat straw and got optimum activity at 40% of initial moisture content [40].

3.8 Effect of Yeast Extract and Peptone Content on Cellulase Activity

Addition of nitrogen source like yeast extract and peptone enhances the secretion of hydrolytic enzymes. In this present study, effect of varying concentrations of yeast extract and peptone was checked on cellulase activity. Increase in concentration of yeast extract from 0.06gm to 0.30gm and concentration of peptone from 0.1gm to 0.5gm, showed increase in cellulase activity. Further increase in concentration of yeast extract to 0.48 gm and peptone concentration from 0.5 to 1.0 gm, decreased cellulase activity. Optimum activity of 7.23IU/min was observed at 0.5 gm of peptone concentration and 7.18IU/min at 0.30 gm of yeast extract concentration as shown in Fig. 8 and 9 respectively.

S. Shafique et al. 2004 reported production of exoglucanase by *Bacillus subtilis* on banana stalk and got optimum activity at 0.1% (w/w) of peptone and 0.4% (w/w) of yeast extract content [37].
4. CONCLUSION

Banana stem waste provides a low cost feed stock for biological production of cellulase. In present study, cellulase produced by Cellulomonas uda (NCIM No.2353) was obtained from NCIM, NCL, Pune. The maximum activity of cellulase produced by Cellulomonas uda on banana waste was recorded for different parameters like particle size, pH, temperature, incubation period, moisture content, peptone and yeast extract content. Experimental setup showed that cellulase production can be done by using banana pseudo-stem; instead of burning them in the agriculture field, which otherwise causes serious environmental concerns by increasing the air pollution. Thus, our research highlighted the use of banana stem waste as potential economic source for the production of cellulase by solid state fermentation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bhati N, Shreya, Sharma AK. Costeffective cellulase production, improvement
strategies, and future challenges. J Food Process Eng. 2020;e13623.
Available:https://doi.org/10.1111/jfpe.13623.

2. Anita Singh a, Somvir Bajar, Arti Devi, Deepak Pant (2021); Bioresource Technology Reports 14. 2021;100652.

3. Pramod B Patil, Payal A Patil, Rutuja R Deshmukh, Sharanappa A, ID Patil; Bioprocessing of Algal Waste for Cellulase Production by *Cellulomonas uda* (NCIM 2353); International Journal of Advanced Biotechnology and Research(IJBR) ISSN 0976-2612. Online ISSN 2278–599X.
2014;5(3):547-551.

4. Bhat MK, Bhat S. Cellulases degrading enzymes and their potential industrial applications. Biotechnology Advance. 1997;15:583-620.

5. Persson I, Tjemeld F, Hohn- Hagerdahl B. Fungal cellulolytic enzyme production: A review. Process Biochem. 1991;26:65-74.

6. Doppel Baurer R, Esterbauer H, Steiner W, Lafferty R, Steinmuller H. The use of cellulosic wastes for the production of cellulases by Trichoderma reesei. Applied Microbial Biotechnology. 1987;26:485-494.

7. Schulein M. Cellulases of Trichoderma reesei. Methods in Enzymology, edited by Wood W A and Abelson J N.(Academic Press, New York). 1988;160:234-242.

8. Xia L, Cen P. Cellulase production by solid state fermentation on lignocellulolic waste from the xylose industry. Process Biochem. 1999;34:909-912.

9. Pooja K Mahale, Desai SV, Hombalimath VS, Sharanappa Achappa (2015); Isolation, screening and characterization of lipase producing strain from soil contaminated soil of Hubballi, Karnataka; International Journal of Basic and Applied Biology. 2015;2(4):198-201.

10. Sharanappa Aachapa, Veeranna S Hombalimath, Jayshree R Kamaraddi, Shivalingsarj V Desai, Anil R Shet, Laxmikant R Patil, Jagadish R Patil. Statistical optimization of lipase production from bacillus species by submerged fermentation. Bioscience Biotechnology Research Communications. 2021;14(1):264-269.

11. Anil R Shet, Laxmikant R Patil, Veeranna S Hombalimath, Sharanappa Achappa. Parametric optimization of oil extraction and lipase catalyzed biodiesel production from rice bran. Journal of Bioscience Biotechnology Research Communications. 2021;14(1):340-345.

12. Hombalimath VS, Desai SV, Sharanappa A. Characterization of lipase immobilized on chitosan Magnetic microparticles for economic biodiesel production. International Journal of Scientific and Technology Research. 2020;9(3):5111-5116.

13. Anil R Shet, Sanjana More, Patil LR, Sharanappa A, Hombalimath VS, GururajTennalli. Immobilization of Xylanase in PVA-Alginate matrix and its characterization. World Journal of Pharmaceutical and Life Science. 2020;6(3):88-91.

14. Veeranna S Hombalimath, Sharanappa Achappa, Laxmikant R Patil, Anil Shet, Shivalingsarj V Desai. Optimization of xylanase production from *Aspergillus* spp. Under solid state fermentation using lemon peel as substrate. Journal of Pharmaceutical Research International. 2021;33(47B):35-43.

15. Laxmikant R Patil, Anil R Shet, Sharanappa Achappa, Shivalingsarj V Desai, Veeranna S Hombalimath, Misba M Kallur. Statistical optimization of media components for xylanase production by *aspergillus* spp. Using Solid State Fermentation and its Application in Fruit Juice Clarification. Journal of Pharmaceutical Research International. 2021;33(54A):151-166.

16. Sharanappa A, Wani KS, Pallavi Patil. Bio processing of food industrial waste for *α*-amyolase production by solid-state fermentation. International Journal of Advanced Biotechnology and Research. 2011;2(4):473-480.

17. Bhavikatti S, Saikrishna Rahul M, Bodduchari, Rahul S Kamagond, Shivalingsarj V Desai, Anil R Shet. Statistical optimization of protease production using a freshwater bacterium *Chryseobacterium cucumeris* SARJS for multiple industrial applications. Journal of 3 Biotech. 2020;10:279.

18. Anil Ramdas Shet, Shivalingsarq Vijay Kumar Desai, Sharanappa Achappa. Pectinolytic enzymes: classification, production, purification and applications. Research Journal of life sciences. Bioinformatics, Pharmaceutical and Chemical sciences. 2018;4(3):337-348, ISSN 2454 – 6348.
19. Gusakov AV, Berlin AG, Popova NN, Okunev ON, Siniltyna AP. A Comparative study of different cellulase preparations in the enzymatic treatment of cotton fabrics. Applied Biochem Biotechnology. 2000;88:119-126.

20. Galante M, Formantici C. Enzyme application in detergency and in manufacturing industries. Curr Org Chem. 2003;7:1399-1422.

21. Kottwitz B, Schambil F. Cellulase and cellulose containing detergent. US Pat. 2005;20050020472.

22. Buchert J, Suurnakki A, Tenhanen M, Viikari L. Enzymatic charaterization of pulps. Enzymes for pulp and paper processing, edited by T W. Jeffries and L Viikari, ACS. Symp ser. 1996:655:38-43.

23. Lewis GE, Hunt CW, Sanchez WK, Treacher R, Pritchard GT, Feng P. Effect of direct fed fibrolytic enzymes on the digestive characteristics of a forage based diet fed to beef steers. J Animal Sci. 1996;74:3020-3028.

24. Galante M, De Conti A, Monteverdi R. Application of Trichoderma reesei enzymes in food and feed industries, in Trichoderma and Gliocladium- Enzymes, Biological control and commercial applications, edited by G. F. Harman and C. P. Kubicek (Taylor and Francis, London). 1998; 2:327-342.

25. Uhlig H. Industrial Enzymes and their applications (John Wiley and sons, INS, New York). 1998:435.

26. Akhtar M. Biochemical pulping of aspen wood chips with three strains of Ceriporiopsis, Subvermispora, Holzforschung, 1994;48:199-202.

27. Prasad DY, Heitmann JA, Joyce TW. Enzyme de-inking of black and white letter press printed news print waste. Prog paper recycle. 1992;1:21-202.

28. Sharanappa A, Anil R Shet, Laxmikant R Patil, Veeresh S Hombalimath, Santosh Kadapure. Biosynthesis of silver nanoparticles using Citrus sinensis peel extract and their application as antibacterial agent. International Journal of Research in Pharmaceutical Sciences. 2020;11(3):4726-4732.

29. Laxmikant R Patil, Anil R Shet, Arati G Lohar, Gururaj B Tennalli, Sharanappa A, Hombalimath V S. Optimization of process parameters for synthesis of silver nanoparticles using leaf extract of tridax procumbens and its biotechnological applications. International Journal of Scientific and Technology Research. 2020;9(6):1050-1056.

30. Anil R Shet, Laxmikant R Patil, Veeranna S Hombalimath, Sharanappa Achappa, Shivaling asarj V Desai, Santosh A Kadapure. Biodegradation of basic yellow auramine o dye using staphylococcus spp. Isolated from Textile Industry Effluent. Bioscience Biotechnology Research Communications. 2021;14(4).

31. Anil R Shet, Shwetha Tantri, Arvind Bennal. Economical biosynthesis of silver nanoparticles using fruit waste. Journal of Chemical and Pharmaceutical Sciences. 2016;9(3):2306-2311.

32. Sudha Rani K, Swamy MV, Seenayya G. Increased ethanol production by metabolic modulation of cellulose fermentation in clostridium thermocellum. Biotech Letter. 1997;8:819-823.

33. Bagewadi Zabin, Desai Shivalingasaraj, Hungund Basavaraj, Muddapur Uday, Sharanappa A. Purification and characterization of thermostable alkaline protease from exiguobacterium aurantiacum ZBB 13. Res. J. Biotech. Vol.16(9):94-101; DOI:https://doi.org/10.25303/169rjbt102111; (2021)

34. Sharanappa Achappa, Patil LR, Hombalimath VS, Anil R Shet. Implementation of project-based-learning (pbl) approach for bioinformatics laboratory course. Journal of Engineering Education Transformations, 2020;33:247-252.

35. Sharanappa A, Patil LR, Hombalimath VS, Deepak Yaraguppi, Anil R Shet. Application of statistics in bioprocess engineering laboratory to reinforce students’ ability in data collection, analysis and interpretation. Journal of Engineering Education Transformations. 2018; Special Issue. ISSN 2349-2473, eISSN: 2394-1707.

36. Laxmikant Patil, Gururaj Bhadri, Shivalingasarj Deasi, Anil Shet, Veeresh Hombalimath. Application of statistical modeling and hypothesis testing to reinforce model validation concepts in bioprocess control laboratory. Journal of Engineering Education Transformations. 2021;34:304-311, eISSN: 2394-1707.

37. Muhammad Irfan, Quertualain Syed, Muhammad Yousaf, Muhammad Nadeem,
Shahjhan Baig A, Saghir Ahmed Jafri. Studies on the pretreatment of wheat straw for improve production of carboxymethyl cellulase by thermophilic Trichoderma viride FBL1 in solid state fermentation. Academia Arena. 2010;2(7).

38. Shafique S, Asgher M, Sheikh MA, Asad MJ. Solid State fermentation of banana stalk for exoglucanase production. International Journal of Agriculture and Biology; 2004.

39. Ikram– Ul- Haq, Kiran Shahzadi, Uzma Hameed, Muhammad Mohsin Javed, Qadeer MA. Solid state fermentation of cellulases by locally isolated Trichoderma harzianum for the exploitation of agricultural by products. Pakistan journal of Biological Sciences. 2006;9(9):1779-1782.

40. Ishtiaq Ahmed, Muhammad Anjum Zia, Haziz Muhammad Nasir Iqbal. Bioprocessing of analyzed wheat straw for enhanced cellulase production through process optimization with Trichoderma viride under SSF. International journal of Biological and life sciences. 2010;6:3.

41. Abo-State MAM, Swelim M, Hammad Al, Gannam RB. Some critical factors affecting cellulases production by Aspergillus terrus Mam F-23 and Aspergillus flavus Mam F 35 under solid state fermentation of wheat straw. World Applied Sciences journal. 2010;9(10):1171-1179.

42. Ezyana Kamal Bahrin, Piong Yeau Seng, Suraini Abd-Aliz. Effect of oil palm empty fruit bunch particle size on cellulase production by Botryosphaeria Sp. under solid state fermentation. Australian Journal of Basic and Applied Science. 2011;5(3):276-280.

43. Pothiraj C, Balaji P, Eyini M. Enhanced production of cellulases by various fungal cultures in solid state fermentation of cassava waste. African journal of Biotechnology. 2006;5(20):1882-1885.

© 2022 Achappa et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/80304