RESEARCH

24(84), October 1, 2014

ISSN 2278–5469 EISSN 2278–5450

Discovery

Kinetic Determining Innovations of Carboxymethyl Cellulase Enzyme Isolated from *Trichophyton terrestre* in Carboxymethyl Cellulose Solution

Mansoor A Lone[1☼], Sanjay Sahay[1], Ravinder Rana[1], Manzoor A Rather[2], Farroq A Dar[2], Firdous A Malla[3], Muzaffar A Reshi[4]

1. Govt. Science & Commerce College, Benazeer Bhopal, M.P. India
2. Department of Biological Sciences, SHIATS Allahabad, U.P. India
3. S.S.S.L. Jain PG College, Vidisha, Bhopal, M.P. India
4. Department of Botany, University of Kashmir, India

☼**Corresponding Author:** Govt. Science & Commerce College, Benazeer Bhopal, M.P. India. E mail ID: ahmadmansoor21@yahoo.com

Publication History
Received: 08 August 2014
Accepted: 19 September 2014
Published: 1 October 2014

Citation
Mansoor A Lone, Sanjay Sahay, Ravinder Rana, Manzoor A Rather, Farroq A Dar, Firdous A Malla, Muzaffar A Reshi. Kinetic Determining Innovations of Carboxymethyl Cellulase Enzyme Isolated from *Trichophyton terrestre* in Carboxymethyl Cellulose Solution. *Discovery*, 2014, 24(84), 110-117

Publication License
This work is licensed under a Creative Commons Attribution 4.0 International License.

General Note
Article is recommended to print as color digital version in recycled paper.

ABSTRACT

The techniques employed for bioconversion of cellulosic biomass to simple sugars by carboxymethyl cellulase have a great industrial importance. Cold-adapted microorganisms serve as potential resource of cold-active carboxymethyl cellulose.In this study, *Trichophyton terrestre,* a rare fungal species in Indian soils, isolated from the rhizosphere of *Juglansregia* L. during winter season was

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

used for the production of carboxymethyl cellulase in carboxymethyl cellulose solution by DNS method at various range of temperatures, using Lineweaver-Burk plot which offers a practical graphical method for the analysis of Michealis-Menten equation, for finding enzyme kinetics, such as Km and Vmax. The enzyme kinetic parameters like maximum activity (Vmax), Km and turnover number were recorded at various concentrations of CMC and different temperatures 4˚ C and 50 ˚ C. The enzyme was found to be tolerant and stable at wide range of temperatures with substantial residual cold-activity which enables this fungal species to survive in extreme environmental conditions of northern India. Such property of carboxymethyl cellulase enzyme has extensive range of applications at industrial scale.

Key words: Carboxymethyl cellulase, *Trichophytonterrestre,* CMC, cold-activity.

1. INTRODUCTION

Human kind has been benefited enormously from the study of microbes since their discovery in the 17th century. However, such benefits have come from the study of just a miniscule fraction of the millions of the species of microbes in the environment (Anne and Ann, 2007). The enormous returns have been particularly derived from those microbes which are able to live in the severe environmental conditions like low or high temperature, extremes of pH, high salt concentrations, nutrient deficient soils, etc. The ability of surviving in such environmental conditions might be related with enzyme stability. Sometimes these microbes are called as the nature's 'master chemists' because an endless variety of chemical compounds are derived from their community. They produce the novel and stable enzymes which function under extreme conditions comparable to those prevailing in various industrial processes. Intense environments always provide a unique resource of microorganisms and novel biocatalysts. *Trichopyton terrestre* was isolated from the rhizosphere of *Juglans regia* L. in the Kashmir valley, India, which has its unique environment throughout the world. Cellulose is the structural polysaccharide in plants and the most abundant biomass on earth. It is composed of β-1, 4-linked glucose units which contains both highly crystalline and amorphous regions (Zhang and Lynd, 2004). Researchers have been stimulated to hydrolyze cellulose to soluble sugars by microorganisms for industrial processes owing to great potentiality of this abundant natural product as an alternative energy source (Coughlan, 1990). Cellulosic materials are converted into soluble sugars by many methods like acid hydrolysis, pyrolysis and by employing enzyme celluloses (Cooney et al., 1978). The acid hydrolysis of cellulosic materials is cheaper than the cellulase hydrolysis, but the former often requires high temperature, pressure and leads to the accumulation of repulsive by-products (Fennington et al., 1982). On the other hand, enzymatic hydrolysis does not have these tribulations. Mostly bioconversion of cellulose by enzymes is employed in the industrial processes to synthesize commercially important products. Cellulase refers to the family of enzymes that work to hydrolyze cellulose. There are numerous microorganisms including both bacteria and fungi which have been found to produce a variety of cellulases for the degradation of cellulose (Miranda et al., 2011), but only a restricted group is capable to produce a sufficient amount of cell-free cellulase, which is proficient in completely hydrolyzing the crystalline cellulose *in vitro*. For the degradation of cellulose, fungi utilize the hydrolytic enzymes viz., exo-cellulase, endo-cellulase, cellobiohydrolase, endoglucanase and P-glycosidase (Bhat, 2000), of which endoglucanases has special significance as it catalyze hydrolysis of internal bonds of cellulose i.e. β- 1,4- glucosidic bonds. Continuing research on *Trichophyton terrestre* indicated that the microorganism has a complete set of cellulase enzymes required for the breakdown of cellulose to glucose (Reese et al., 1950). Cellulase enzymes including endoglucanases indicate the potentiality of successful conversions of waste cellulose into foods for our growing population, thus the enzyme exploitation subject demands the intense research even at molecular level (Eveleigh, 1985).

2. MATERIALS AND METHODS

Isolation and identification

Soil samples were collected from 20 cm deep the rhizosphere of *Juglans regia L. randomly in aseptic manner i*n the Northern regions of India (Kashmir valley) during three different seasons-rainy, spring and winter. Fungal colonies were isolated by serial dilution method to get more manageable results (Aneja, 2005). 1g of soil was transferred to 10 ml of distilled water in test tubes. Dilutions were made up to 10^{-6}. Czapek-Dox agar medium was used as culturing media with composition of (g/l); sucrose- 30, $NaNO_3$- 3, K_2HPO_4- 1, $MgSO_4$- 0.5, KCl- 0.5, $FeSO_4$- 0.01, agar agar- 15, pH of the medium was adjusted to 7.3. After autoclaving at 121°C and 15 lbs pressure, 20 ml of sterile medium was transferred to sterile petri-plates (Chloramphenicol 250mg/100ml was also added to check the bacterial growth). 0.1 ml of soil suspension was spread with the help of spreader and incubated at 28˚C for 7 days. The fungal cultures grown on the medium were transferred on to the Potato Dextrose Agar (Hi Media) medium and pH was maintained at 5.6 for further studies. Identification of fungi was done based on cultural, morphological and microscopic characters.

discovery

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

Cellulase production

Among the identified cultures, *Trichophyton terrestre* was selected. A volume of 100 ml of Czapek-Dox broth medium amended with 1% cellulose was distributed into separate 250 ml Erlenmeyer conical flask. The pH of the medium was adjusted to 5. After autoclaving at 121˚C and 15 lb pressure, the fungal spore suspension was inoculated into the conical flask with the inoculum concentration of $1x10^6$ spores mL^{-1}. The flask was incubated at 32°C on a rotary shaker at 120 rpm for 7 days (Lone et al., 2012). After incubation, the content of the flask was passed through Whatman filter paper No.1 to separate the mycelial mat from culture filtrate. The filtrate was used for the estimation of extracellular protein content and total activity of endoglucanases.

Protein estimation

Protein content of the supernatant secreted by fungus was estimated according to Lowry et al., (1951). The experimental data was collected and glucose standard curve (Figure 1) was used as a standard (Kondo et al., 1994).

Kinetics of enzyme endoglucanases

The enzyme endoglucanase in the crude extract was assayed with increasing concentrations of carboxymethyl cellulose (0.16-0.83mg/0.5ml) at 4 ˚C and 50 ˚C at pH 6.5. Enzyme catalyzed reactions often exhibit a special form of kinetics, called Michealis-Menten kinetics, which are characterized by hyperbolic relationship between reaction velocity (V) and substrate concentration (S). Normal enzyme kinetic values are measured under steady-state conditions and such conditions usually prevail in the cell. For many enzyme- catalyzed reactions, the kinetics under steady-state can be described by a simple expression known as Michealis-Menten kinetics:

V = Vmax S / Km+S

Where, V is the observed rate of velocity, Vmax is the maximum velocity (at infinite substrate concentration), S is the substrate concentration and Km is the Michaelis-Menten constant which represents the concentration of substrate required to yield half of the maximum velocity of enzyme catalysis. Smaller the value of Km, more strongly the enzyme binds to substrate. The Lineweaver-Burk plot was used to determine the important kinetics parameters of endoglucanase enzyme such as Km and Vmax (Lineweaver and Burk, 1934). The computerized curve was obtained by fitting the values 1/S on x-axis and 1/Vmax on y-axis (Table 1). The y-intercept of such a graph denotes 1/Vmax, while the x-intercept represent -1/Km. The turnover number, the number of substrate molecules converted into products per unit time per molecule of enzyme, was also obtained by dividing the values of Vmax by the amount of enzyme used in the experiment (Table 2). Typical turnover number values ranges from 10^2 to 10^3 $S.^{-1}$

Table 1

Cellulase activity (µmol /mg protein) of *T. terrestre* at 4°C and 50°C from various CMC concentrations

S No.	CMC (mg/0.5ml)	1/S	Cellulase activity (µmol /mg protein)		1/V	
			4˚C	50˚C	4˚C	50˚C
1	0.16	6.25	25	32.00	0.04	0.0312
2	0.33	3.00	31.5	37.50	0.0317	0.0266
3	0.50	2.00	46.25	53.12	0.0216	0.0188
4	0.66	1.50	50	66.25	0.0210	0.0150
5	0.83	1.20	52.50	69.37	0.019	0.0144

Table 2

Turnover Number of enzyme cellulase at 4˚C and 50˚C temperature

S No.	CMC (mg/0.5ml)	Turnover Number (mol/0.5ml enzyme/30min)	
		4˚C	50˚C
1	0.16	50	64
2	0.33	63	75
3	0.50	92.5	106.24
4	0.66	100	135.50
5	0.83	105	138.74

discovery

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

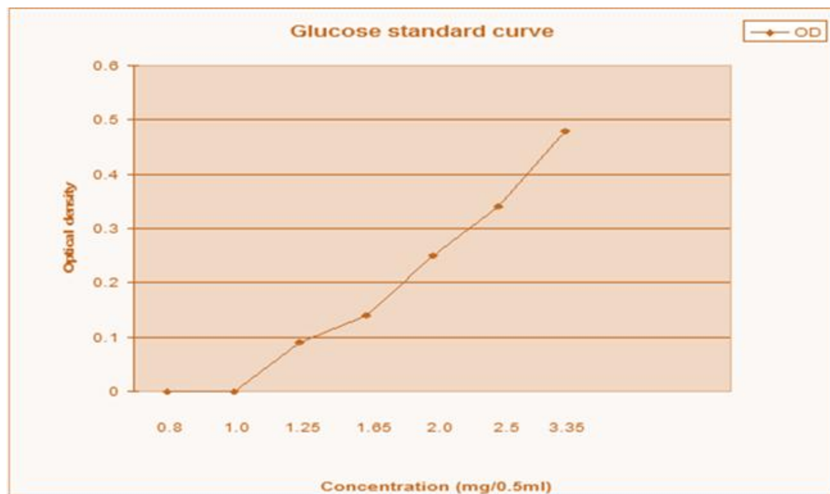

Figure 1

Glucose standard curve

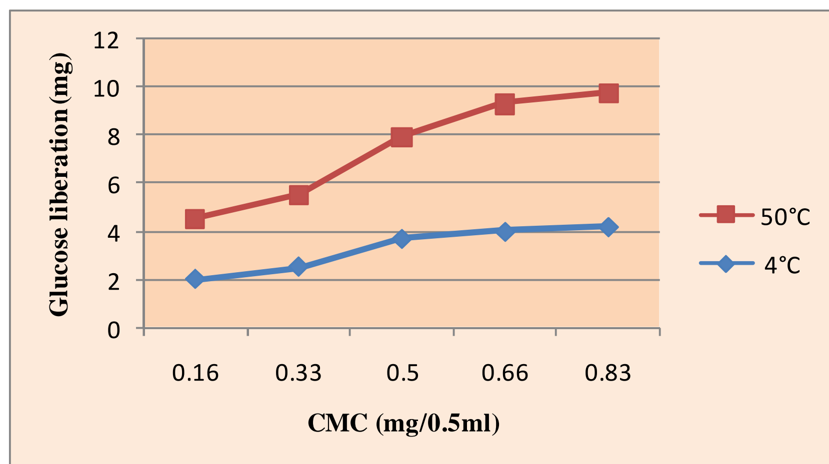

Figure 2

Glucose liberation (mg) at 4°C and 50°C with increasing concentration of CMC (mg/0.5ml)

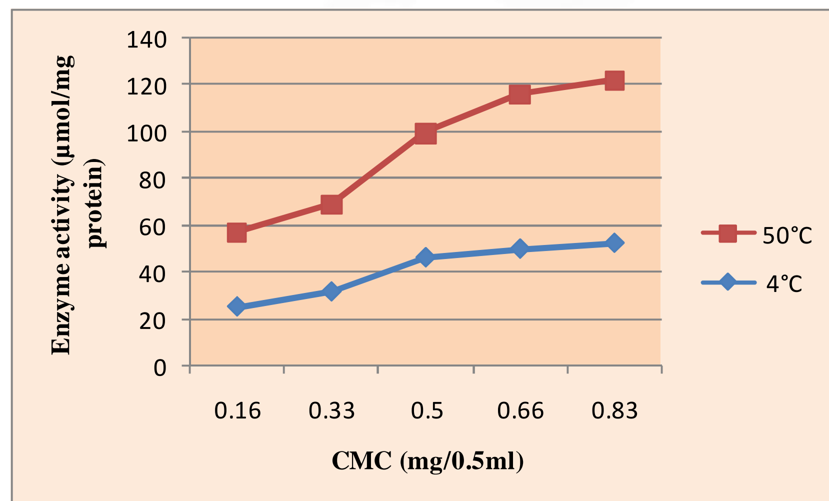

Figure 3

Enzyme activity (µmol/mg protein) at 4°C and 50°C with increasing concentration of CMC (mg/0.5ml)

3. RESULTS AND DISCUSSION

Among an array of fungal strains isolated during rainy, spring and winter seasons from the rhizosphere of *Juglans regia* L., geophilic fungus *Trichophyton terrestre* was isolated only during the winter season and screened for the production and activity of the enzyme endoglucanase. The enzyme secreted by the fungal species in culture solution at 32˚C was purified to homogeneity. All enzymes that act upon the cellulose are deemed to move about the surface in a random walk (Nimlos et al., 2007; Ting et al., 2009) in steps equating to the dimensions of one glucose molecule (5 A) per time-step. The activity of the enzyme was assayed in the carboxymethyl cellulose solution with the increasing substrate concentrations. Glucose production by enzyme participation was determined at 4 ˚C and 50 ˚C by DNS method thereby generating a reddish brown colour for amino compounds (Vancov and Keen, 2009). The glucose liberation in enzymatic reaction with the chromogenic agents occur in the reaction. The absorbance was measured by the spectrophotometeric method at the wavelength of around 540 nm (Figure 2) (Coleman et al., 2007). Enzyme activity with 0.83mg/0.5ml CMC concentration was found to be 52.5 |mol /mg protein and 69.37 |mol /mg protein at 4 ˚C and 50 ˚C respectively (Figure 3). The enzyme remained active at a wide range of temperatures. A broad temperature optimum was observed for the enzyme endoglucanase ranging from 4 ˚C to 50 ˚C. However, the enzyme has been found to be active at 100 ˚C (Nataraja et al., 2010). The turnover number was observed at varied CMC concentrations. The maximum turnover number at 4 ˚C and 50 ˚C was found to be 105mol/0.5ml enzyme/30minutes and 138.74 mol/0.5ml enzyme/30minutes respectively (Figure 4), when the concentration of CMC was 0.83 mg/0.5ml.

The Km value of enzyme endoglucanase was determined at 4 ˚C and 50 ˚C which was found to be 0.312mM and 0.67mM respectively (Figure 5, 6). Being the fingerprint of an enzyme, Km value is considered the most important criterion to evaluate the enzyme for various uses. Km value indicates the higher efficiency of the enzyme. Lesser the Km value, higher the efficiency of an enzyme. The enzyme was found to be more active at 4˚C temperature, when the concentration of CMC was 0.83mg/0.5ml. Intercept or 1/Vmax at 4 ˚C and 50 ˚C was found to be 0.014

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

min/mM and 0.010min/mM respectively. The slope (Km/Vmax) was found to be 0.0043mM/min and 0.0067 mM/min at 4 ˚C and 50 ˚C respectively. The maximum activity (Vmax) determined at 4 ˚C and 50 ˚C was 71.42 |mol/mg protein and 100 mol/mg protein respectively. The determination of the kinetic parameters for CMC hydrolysis reveals a fascinating phenomenon of kinetic optimization at different temperatures. The activity of the enzyme at low and high temperature can be viewed as the main physiological adaptation to cold and high temperatures at the enzyme level, as it compensates for the reduction of chemical reaction rates induced by low and high temperatures (Feller and Gerday 2003; Carrard et al., 2000). Because of the extreme molecular stability of endoglucanase at wide array of temperatures, *Trichophyton terrestre* forms an interesting enzyme source for industrial applications such as in textile industries (Gusakov et al., 2000; Belghith et al., 2001), detergents industries (Maurer, 1997), pulp and paper industry (Buchert et al., 1996), food industry (Galante et al., 1998), and in improving digestibility of animal feeds (Lewis et al., 1996). The enzyme thus accounts for a significant share of the world enzyme market. In addition to the above applications, endoglucanses are also employed in generation of antibacterial chito-oligosaccharides which may well be used in food preservation (Liu and Zhu, 2000), immuno-modulation (Tsai et al., 2000) and as potent antitumor agents (Wu and Tsai, 2004). In the biocatalyzed-reaction networks, inhibitory effects of the reaction intermediates play an important role in determining the enzymatic kinetics. The intrinsic reaction kinetics of enzymatic cellulose hydrolysis is also subjected to mediation by a host of factors like inhibitory effects of reaction intermediates and enzyme adsorption etc. The soluble products such as cellobiose and glucose have been reported to be the inhibitors of the cellulase complex (Howell and Stuck, 1975; Katz and Reese, 1968; Ghose, 1977; Nisizawa, 1973), and individual enzyme endoglucanase components (Halliwell and Griffin, 1973; Ladisch et al., 1980), cellobiohydrolase (Hsu et al., 1980) and p - glucosidase (Wood and Mc Care, 1975; Gong et al., 1997). Furthermore, p-glucosidase is also inhibited by its substrate cellobiose (Emert et al., 1974; Berghem and Pettersson, 1974). In recent years, the potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Akpan et al., 1999; Abu et al., 2005). The activities of endoglucanses from *Trichophyton terrestre* were greatly influenced by the concentration of the substrate. During the initial stage of hydrolysis cellulose released more amount of glucose to the solution (Andrew et al., 2011). It is obvious that the concerned mechanisms are extremely multifaceted, since the enzyme effect coupled with intrinsic properties of substrate such as degree of polymerization, crystallinity, or accessible surface area which play crest roles in the enzymatic degradation. The optimal pH of 6.5 for the endoglucanse activity is in conformity to that of Catriona *et al.*, who reported a broad pH range of 5.0 to 7.0 over which the cellulases were highly active. Bok *et al.*, (1998) also reported a pH range of 6.0 to 6.6 for two thermostable endocellulases from *Thermotoga neapolitana*. The physicochemical or ecological conditions existing during the enzyme action need to be taken into account to gain a better understanding of any experimentation. Specifically, the substrate composition, crystallinity, and recalcitrance (Fierobe et al., 2002; Himmel et al., 2007; Jeoh et al., 2006) have a distinctive influence on the mechanism and dynamics of cellulose strap and hydrolysis (Woodward et al., 1992; Be'guin and Aubert, 1994). Cellulases especially endoglucanses isolated from different sources have a wide range of properties depending on their sources. Industrial enzymes working at different temperatures allow technologists to develop processes which closely approach the gentle and efficient processes in nature. The biocatalysts which remain active at cold conditions find their place in chemical synthesis and transformation, bioremediation of contaminants and clean-energy production, confirming and reinforcing the potential of this technology for environmental purposes. The maximum activity (Vmax) was determined at wide range of temperatures. The most important finding was the extraordinary level of tolerance at low and higher temperatures along with a great turn over number. The enzymes showing activity at wide range of temperatures are today the enzymes of choice for microbiologist, biochemists, biotechnologists and industrialists (Kazem, 2012). Endoglucanases active at low and high temperatures are promising enzymes to reinstate the conventional enzyme processes. There is an immediate need for the selection of ideal endoglucanses to retain the activity at extreme range of temperatures which may be utilized in a variety of fields in order to obtain the best results. In this direction, *Trichophyton terrestre* could be used as a prospective source of the enzyme. Extensive research is vital to unknot the full potential of such micro-organisms.

4. CONCLUSION

The present study, confirmed that the endoglucanases isolated from *Trichopyton terrestre* can tolerate a wide range of temperatures 4˚C and 50˚C which enables this fungal species to survive in the extreme environmental conditions of northern India, where the winter temperature goes down to -20˚C and touches the upper limit of 37˚C in the summer season. Such a great fluctuation in the temperature occurs only in few regions of India which might be the reasonable factor for the restricted distribution of *Trichophyton terrestre* in Indian soils. The biocatalysts which remain active at cold and hot conditions are desirable in industrial processes. However, an extensive research work is required to overcome several bottlenecks such as less explored biodiversity of psychrophilic

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com OPEN ACCESS

and thermophilic microbes, low activity and stability in terms of turnover number of enzymes under varied environmental conditions.

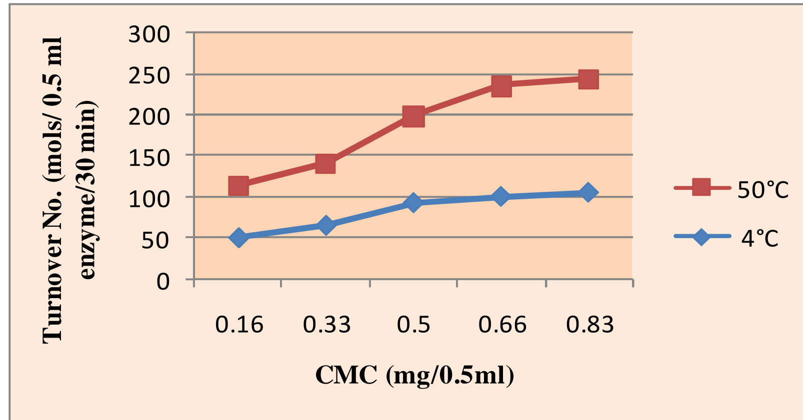

Figure 4

Turnover number (mols/ 0.5 ml enzyme/30 min) at 4°C and 50°C with increasing concentration of CMC (mg/0.5ml)

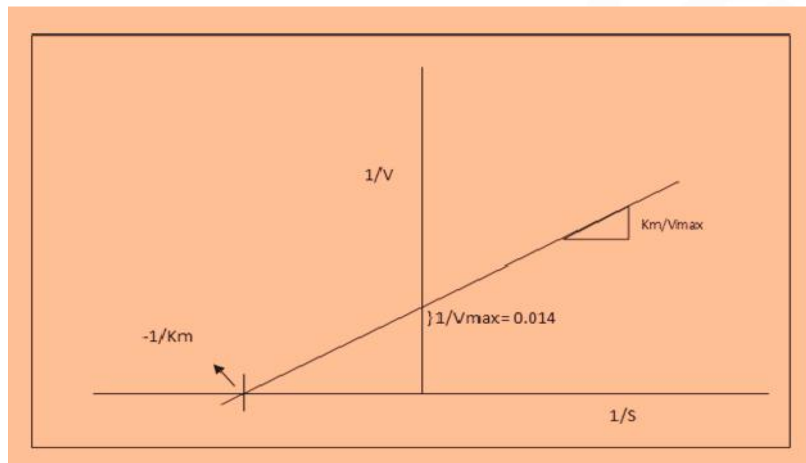

Figure 5

Km determination at 4°C

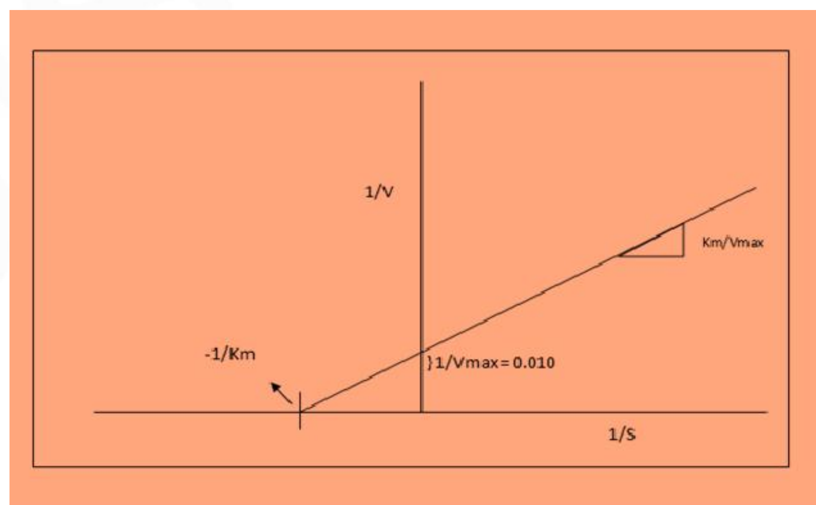

Figure 6

Km determination at 50°C

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

ACKNOWLEDGEMENT

The authors are gratified to the Principal, Govt. Science and Commerce College Benazeer, Bhopal- 462 008 (MP), India for providing all the requisite infrastructural support and laboratory facilities with regard to complete these experiments, which were purely funded by authors.

REFERENCE

1. Abu EA, Ado, SA, James, DB. Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on *Sorghum pomace. African Journal of Biotechnology,* 2005, 4: 785-790
2. Akpan I, Bankole MO, Adesemowo AM and Latunde-Dada GO. Production of amylase by *A. niger* in a cheap solid medium using rice band and agricultural materials. *Tropical Science,* 1999, 39: 77-79
3. Andrew CW, Bryce AL, Victoria SH . A cellular automaton model of crystalline cellulose hydrolysis by cellulases. *Biotechnology & Biofuel,* 2011, 4-39
4. Aneja KR. Experiments in microbiology, plant pathology and biotechnology, 4th edn. New Age International Pvt. Ltd., India, 2005
5. Anne J, Ann R. The new science of metagenomics: revealing the secrets of our microbial planet. *National Academic Press*, 2007, 624-6242
6. Be'guin P, Aubert J. The biological degradation of cellulose. *FEMS Microbiology*, 1994, 13: 25-58
7. Belghith H, EllouzChaabaouni S, Gargouri A. Biostoning of denims by *Penicillium occitanis*Cellulases. *Journal of Biotechnology,* 2001, 89: 257-262.
8. Berghem LER, Pettersson LG. The Mechanism of enzymatic cellulose degradation isolation and some properties of a P-Glucosidase from *Trichoderma viride. European Journal of Biochemistry*1974,46: 295-305
9. Bhat MK. Cellulases and related enzymes in biotechnology. *Biotechnology Advances,* 2000, 18: 355-383
10. Bok JD, Yernool DA, Eveleigh DE. Purification, characterization and molecular analysis of thermostable cellulasescel A and cel B from *Thermotoga neapolitana. Applied Environmental Microbiology,* 1998, 6: 4774-4781
11. Buchert J, Suurnakki A, Tenkanen M, Viikari L. Enzymatic characterization of pulps, In: Enzymes for pulp and paper processing, edited by Jefferies TW, Viikari L, Symp ACS, 1996, 655: 38-43
12. Carrard G, Koivula A, Soderlund H, Beguin P. Cellulose-binding domains promote hydrolysis of different sites on crystalline cellulose. *Proceedings of the National Academic of Science* U.S.A, 2000, 97: 10342-10347
13. Catriona AW, Sheila IM, Thomas MW. Characterization of a p-D-glucosidase from the anaerobic rumen Fungus *Neocallimastix rontalis* with particular reference to attack on cello-oligosaccharides. *Journal of Biotechnology,* 1994, 37: 217-227
14. Coleman DJ, Studler MJ, Naleway JJ. A long-wavelength fluorescent substrate for continuous engineering plants and enzymes for biofuels production. Science (Washington, DC, U.S., 2007, 315: 804-807
15. Cooney CL, Wang DIC, Wang SD, Gordon J, Jiminez M. Simultaneous cellulose hydrolysis and ethanol production by a cellulolytic anaerobic bacterium. *Biotechnology and Bioengineering,* 1978, 8: 103-114
16. Coughlan MP. Cellulose degradation by fungi. In: Fogarty WM, Kelly CT (ed) *Microbial enzymes and biotechnology,* 2ndedn. *Else. Appl Sci*, London: United Kingdom, 1990, 1-36
17. Emert GH, Gum EK, Lang JA, Lin TH, Brown RD. Cellulases: in food related enzymes. *Advances in Chemistry,* 1974, 136: 79-100.
18. Eveleigh DE. *Trichoderma* in Biology of industrial micro-organisms, Eds, Demain AL & Solomon NA. Menlo Park, C.A: Butterworths, 1985, 487-509
19. Feller G, Gerday C. Psychrophilic enzymes: hot topics in cold adaptation. *Nature Reviews Microbiology,* 2003, 1: 200-208
20. Fennington G, Lupo D, Stutzenberger F. Enhanced cellulase production in mutants of *Thermomonospora curvata. Biotechnology and Bioengineering,* 1982, 24: 2487-2497
21. Fierobe H, Bayer E, Tardif C, Czjzek M, Mechaly A, Belaich A, Lamed R, Shoham Y, Belaich J. Degradation of cellulose substrates by cellulosome chimeras: substrate targeting versus proximity of enzyme components. *Journal of Biology and Chemistry,* 2002, 277: 49621-49630
22. Galante YM, De Conti, Monteverdi R. Application of *Trichoderma* enzymes in food and feed industries, In: *Trichoderma & Gliocladium Enzymes,* biological control and commercial applications, edited by Harman GF &KubiceSk CP (Taylor & Francis, London), 1998, 2327-342
23. Ghose TK, Fiechter N, Blakeblough. Cellulase biosynthesis and hydrolysis of cellulosic substances. *Advances in Biochemistry and Engineering*, 1977, 39-76
24. Gong CS, Ladisch MR, Tsao GT. Cellobiase from *Trichoderma viride:* purification, properties, kinetics, and mechanism. *Biotechnology and Bioengineering,* 1997, 19: 959-81
25. Gusakov AV, Berlin AG, Popova AN, Okunew ON, Sinitsyna AP. A comparative study of different cellulase preparations in the enzymatic treatment of cotton fabrics. *Applied Biochemistry and Biotechnology,* 2000, 88: 119-126
26. Halliwell G, Griffin M. The nature and mode of action of the cellulolytic component C_1 of *Trichoderma koningii*on native cellulose. *Biochemistry Journal,* 1973, 135: 587-594

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

27. Himmel M, Ding S, Johnson D, Adney W, Nimlos M, Brady J, Foust T. Biomass recalcitrance: fluorometric determination of cellulase activity: resorufin-p-D-cellobioside. *Annals of Biochemistry*, 2007, 371: 146-153
28. Howell JA, Stuck JD. Kinetics of solkafloc cellulose hydrolysis. *Biotechnology and Bioengineering,* 1975, 17: 873-893
29. Hsu TA., Gong CS, Tsao GT. Kinetic studies of cellodextrins hydrolysis by exocellulase from hydrolysate with immmunoactivity. *Fisheries Science,* 2004, 70: 1113
30. Jeoh T, Wilson D, Walker L. Effect of cellulase mole fraction and cellulose recalcitrance on synergism in cellulose hydrolysis and binding. *Biotechnology Progress,* 2006, 2: 270-277
31. Katz M, Reese ET. Production of glucose by enzymatic hydrolysis of cellulose. *Applied Microbiology,* 1968, 16:419-420
32. Kazem K. Hyperthermophiles: metabolic diversity and biotechnological applications. In: Extremophiles: *microbiology and biotechnology*. Roberto Paul Anitori (Eds) Caister Academic Press, 2012, 206
33. Kondo A, Urabe T, Higashitani K. Bioconversions in aqueous two-phase system using enzymes immobilized on ultrane silica particles. *Journal of Fermentation and Bioengineering*, 1994, 77: 700-703
34. Ladisch MR, Gong CS, Tsao GT. Cellobiose hydrolysis by endoglucanase (glucan-glucano hydrolase) from*Trichoderma reesei:* kinetics and mechanisms. *Biotechnology and Bioengineering,* 1980, 22: 1107-1126
35. Lewis GE, Hunt CW, Sanchez KW, Treacher R, Pritchard G T, Feng P. Effect of direct- fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. *Journal of Animal Science,* 1996, 74: 3020-3028
36. Lineweaver H, Burk D. The determination of enzyme dissociation constants. *Journal of American Chemistry Society,* 1934, 56: 658-666
37. Liu W, Zhu WM. Production and regeneration of *Trichosporon cutaneum* protoplasts. *Process Biochemistry,* 2000, 35: 659-664
38. Lone MA, Wani MR, Bhat NA, Sheikh SA, Reshi MA. Evaluation of cellulases enzyme secreted by some common and stirring rhizosphere fungi of *Juglans regia* L. by DNS method. *Journal of Enzyme Research,* 2012, 3: 18-22
39. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. *Journal of Biology and Chemistry,* 1951, 193: 265-75
40. Maurer KH. Development of new cellulases, In: Enzymes in Detergency, edited by Jan H Van E et al., (Marcel Dekker, New York), 1997, 175-202
41. Miranda LM, Michael B, Kam TL, Wensheng Q. Characterization of some efficient cellulase producing bacteria isolated from paper mill sludges and organic fertilizers. International Journal of *Biochemistry and Molecular Biology,* 2011, 2: 146-154
42. Nataraja S, Chetan DM, Krishnappa M. Effect of temperature on cellulose enzyme activity in crude extracts isolated from solid wastes microbes. *International Journal of Microbiology Research,* 2010, 2: 44-47
43. Nimlos MR, Matthews JF, Crowley MF, Walker RC, Chukkapalli G, Brady JW, Adney WS, Cleary JM, Zhong L, Himmel ME. Molecular modeling suggests induced fit of Family I carbohydrate-binding modules with a broken-chain cellulose surface. *Protein Engineering Design and Selection,* 2007, 20: 179-187
44. Nisizawa K. Mode of action of cellulases. *Journal of Fermentation Technology,* 1973, 51: 267-304
45. Reese ET, Sui RGH, Levinson HS. The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis. *Journal of Bacteriology,* 1950, 59: 485-497
46. Ting CL, Makarov DE, Wang ZG. A kinetic model for the enzymatic action of cellulase. *Journal of Physical Chemistry B,* 2009, 113: 4970-4977
47. Tsai GJ, Wu ZY, Su WH. Antibacterial activity of a chitooligosaccharide mixture prepared by cellulose digestion of shrimp chitosan and its application to milk preservation. *Journal of Food Protection,* 2000, 63: 747-752
48. Vancov T, Keen B. Rapid isolation and high-throughput determination of cellulase and laminarinase activity in soils. *Journal of Msicrobiology Methods,* 2009, 79: 174-177
49. Wood TM, Mc Care ST. Symposium on enzymatic hydrolysis of cellulose. Eds., Helsinki, Finland, 1975, 231
50. Woodward J, Affholter KA, Noles KK, Troy NT, Gaslightwala SF. Does cellobiohydrolase II core protein from *Trichoderma reesei* disperse cellulose macrofibrils. *Enzyme Microbiology and Technology,* 1992, 14: 625-630
51. Wu GJ, Tsai GJ. Cellulase degradation of shrimp chitosan for the preparation of water-soluble *Trichoderma reesei. Biotechnology and Bioengineering*, 1980, 22: 2305-2320
52. Zhang YHP, Lynd LR. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. *Biotechnology and Bioengineering,* 2004, 88: 797-824

© 2014 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS