INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–652/2014/16–6–1141–1146
http://www.fspublishers.org

Full Length Article

Endoglucanase Production by *Humicola insolens*: Effect of Physiochemical Factors on Growth Kinetics and Thermodynamics

Hina Riaz[1,2], Abdul Jabbar[2], Muhammad Hamid Rashid[1*], Sana Riaz[2] and Farooq Latif[1]
[1]*Enzyme Engineering Lab., I.B. Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), P.O. Box 577, Jhang Road, Faisalabad, Pakistan*
[2]*Department of Chemistry, GC University, Faisalabad, Pakistan*
*For correspondence: hamidcomboh@gmail.com

Abstract

The effect of physiochemical factors on the productivity of endoglucanases by a local thermophillic fungal strain of *Humicola insolens* was investigated. Carboxymethylcellulase (CMCase) was produced under submerged fermentation and the effects of inoculum size, temperature, pH and substrate concentration on the growth kinetic parameters (μ, t_d, $Y_{p/x}$, q_p, μ_{max}, $q_{p(max)}$ were determined. Optimal conditions for CMCase production were: inoculum density= 8% v/v (12.41mg cells mL^{-1}), substrate (CMC) = 5% (w/v), pH = 5.0, temp = 45°C. The extracellular CMCase and protein production under optimized conditions was 2.0 UmL^{-1} and 0.129 mg mL^{-1}, respectively. Growth kinetic parameters were as follows: specific growth rate (μ) = 0.046; t_d = 15.02 h; $Y_{p/x}$ = 0.0139, μ_{max} = 0.0458 h^{-1}, $q_{p(max)}$= 0.0011 U h^{-1} mg^{-1} cell mass. The activation energy for the enzyme production $E_{a(p)}$ was 108.69 kJ mol^{-1}. Thermodynamic parameters i.e., ΔH^*_p, ΔG^*_p and ΔS^*_p were 106.047 KJ mol^{-1}, 117.589 KJ mol^{-1} and -36.29 J $mol^{-1}K^{-1}$, respectively. The results are novel because it is the first report on kinetics and thermodynamics of CMCase production by *H. insolens*. © 2014 Friends Science Publishers

Keywords: CMCases; Gibbs energy; Entropy; Enthalpy; Activation energy

Introduction

Enzymes enhance availability of nutrients from the feedstuffs, decrease the cost of feed and reduce the waste out put into the environment. The enzymes have a long history for their use in various industries like: textile, leather, pharmaceutical, baking, brewing, detergents, etc. but their use in the livestock feed has found to be a current phenomenon. Cellulases and amylases can increase the feed digestibility, resulting into improvement in performance of animals and the energy value of ingredients (Bhatti *et al.,* 2013). Cellulases have been widely used in food and pharmaceutical industries and also for controlling environmental pollution (Jabbar *et al*., 2008; Niaz *et al.*, 2011; Huma *et al.*, 2012).

According to Economic Survey of Pakistan, livestock contributes about 11.9% of Pakistan's GDP, which is more than the crop sector. Furthermore, the production of beef and mutton in Pakistan during 2012-2013 was 1.829 and 0.643 million tons, respectively (GOP, 2013). Poultry sector is one of the most organized and vibrant segments of the agriculture industry of Pakistan. This sector generates employment (direct/indirect) and income for about 1.5 million people. Its contribution in agriculture is 5.76%, livestock 10.4% and in GDP at constant cost factor 1.2%. Poultry meat contributes 26.8% of the total meat production in the country (GOP, 2013).

Various additives, such as, plant extracts or powders, enzymes, vitamins etc. are mixed in the poultry feed to improve the performance of animals. Furthermore, they improve the quality of the poultry litter, which has great application in the agriculture sector (Duru, 2012; Reddy *et al*., 2012).

Endoglucanases or carboxymethylcellulases (ß-1,4-D-glucan-4-glucanohydrolases, EC 3.2.1.4) belong to cellulase system. The other members of cellulases include exoglucanases (EC. 3.2.1.91) and cellobiases (EC. 3.2.1.21), which act in synergy (Jabbar *et al*., 2008). Cellulose is the major component of plant biomass. It is a linear, un-branched homopolysaccharide consisting of glucose subunits joined together by ß 1-4 glycosidic linkages and its molecules (polymer) vary widely in length and are usually arranged in bundles or fibrils (Haruta *et al*., 2002; Walsh, 2002). The crystalline structure of cellulose is disrupted by endoglucanases, which breaks the internal bonds; as a result the individual cellulose polysaccharide chains are exposed (Walsh, 2002). Afterwards, from the ends of these chains 2-4 units are cleaved by exoglucanase. As a result tetrasaccharides or disaccharides like cellobiose are produced. The cellobiose is hydrolyzed by β-glucosidase or Cellobiase into individual monosaccharides (Javed *et al.*, 2011; Khan and Singh, 2011).

To cite this paper: Riaz, H., A. Jabbar, M.H. Rashid, S. Riaz and F. Latif, 2014. Endoglucanase production by *Humicola insolens*: Effect of physiochemical factors on growth kinetics and thermodynamics. *Int. J. Agric. Biol*., 16: 1141–1146

Cellulose degrading enzymes are extensively produced today using submerged growth conditions by various microbes including bacteria, fungi and protozons (Lynd *et al*., 2002). Among microbes, fungi have greatest potential to produce cellulases (Irfan *et al*., 2011). Many filamentous fungi have been reported to produce cellulases including *Trichoderma ressei, T. viride, Aspergillus niger, Arachniotus citrinus, Gymnoascella citrine, Humicola* species (Jabbar *et al*., 2004, 2008; Leite *et al.*, 2008).

Cellulases have wide application in food, textile, pharmaceutical and livestock feed industries. The enzymes offer a unique environment friendly way to improve the digestion of feed with enhanced feed conversion and manure quality. Hence, results in improvement in animal's health. Almost whole enzyme demand of Pakistan is fulfilled through import from foreign countries.

In Pakistan, enzymes usage in poultry has increased dramatically, but conversely their use in larger animal feed is practically negligible because price of cattle feed is low, while that of enzymes is very high. Almost whole enzyme demand of Pakistan is fulfilled through import from foreign countries. It involves an enormous investment of foreign exchange, which is an immense burden on the lean economy of Pakistan. To develop appropriate enzymes, Pakistan needs extensive research and development work and for the production of economically feasible enzymes, industrial scale enzyme production units must be installed in the near future.

The information about physiochemical properties of microbes such as effect of hydrogen ion concentration (pH), temperature, substrate concentration, inoculums density etc. on the production of enzymes is essential for the up-scaling of the process at pilot level. The information on growth kinetic and thermodynamic parameters further helps to evaluate the economic feasibility of microbial enzyme production at pilot scale.

Here we report, for the first time about growth kinetics and thermodynamics for the CMCases production by local thermophillic fungal strain of *H. insolens* under submerged fermentation. Moreover, the envisaged project deals with the effect of temp, pH, substrate and inoculum density on the production of endoglucanases.

Materials and Methods

The experiments were done in *Enzyme Engineering Laboratory*, I.B. Division, NIBGE, Faisalabad. The chemicals used were of analytical grade and were mainly purchased from MP Biomedical, Sigma chemical Company, USA etc.

Microbial Strain and Culture Maintenance

Local isolate of *H. insolens* (Cooney and Emerson, 1964; Ellis, 1982) was obtained from NIBGE, Faisalabad (Luqman *et al*., 2010). The culture was maintained on potato-dextrose agar media (PDA) slants and Petri plates. Conidia of *H. insolens* were single or a short chain of 2-3 spores, with stalk or without, while, the spores color at maturation was first white, then mouse gray and finally deep mouse gray (Fig. 1).

Inoculum Preparation and Biomass Estimation

Inoculum was prepared using Eign and Push medium [KCl = 0.5 g/L, $(NH_4)_2SO_4$ = 0.5g /L, $MgSO_4$ = 0.2 g/L, $CaCl_2$ = 0.1 g/L, KH_2PO_4 = 1 g/L, Yeast extract = 0.5 g/L, Urea = 20% and glucose = 2%]. Salts were weighed and mixed with distilled water one by one to avoid precipitation in Erlenmeyer flask. The pH of medium was 5.0 and volume was made with distilled water (80 mL/250 mL flask). Five glass beads were added in each flask to form uniform suspension by breaking mycelial pellets. The flasks were plugged with cotton, covered with aluminum foil and autoclaved at 121°C and 1.05 kg/cm^2 pressure. The test flask containing autoclaved Eign and Push medium was inoculated with a loop full of *H. insolens* spores under aseptic conditions and were allowed to grow at 45°C on a rotary shaker for 72 h.

Biomass or cell mass content was estimated based on spectrophotometry by measuring the light transmitted at 610 nm (Pirt, 1975). Cell mass standard curve of *H. insolens* was prepared, which was used to measure the inoculums strength, so that uniform inoculum transfer to the growth medium was ensured (Nadeem *et al*., 2009; Javed *et al.*, 2011).

CMCase Assay

100 µL of CMCase was mixed in reaction mixture containing: 1 mL Carboxymethylcellulose sodium salt (CMC) solution [1.0% CMC (w/v)] + 1 mL 50 mM sodium acetate buffer, pH 5.0 and incubated for 40 min at 50°C. Quenching of the reaction was done by placing the tubes in boiling water bath for 5 min. The released reducing sugars were determined by DNS method and glucose was used as a standard (Huma *et al.*, 2012). Total volume of DNS assay i.e. for the color development was 4.1 mL (2 mL DNS reagent + 1 mL quenched reaction mixture + 1.1 mL distilled water). The reaction mixture was boiled for 10 min and after cooling absorbance was taken at 550 nm.

One unit of CMCase activity was defined as "µmol of glucose equivalents liberated per min under defined reaction conditions". The units were calculated by using the following formula:

$$U\ ml^{-1}\ min^{-1} = \frac{\Delta A_{550}\ \text{of sample} \times \text{Glucose standard factor (3.01 µmol)} \times \text{Total reaction mixture (2.1 ml)}}{\text{Enzyme (0.1 ml)} \times \text{Time (40 min)} \times \text{Reaction mixture used for colour development (1.0 ml)}}$$

Protein Estimation

Total protein was determined by Bradford method and bovine serum albumin (BSA) was used to prepare the calibration curve as described by Riaz *et al.* (2012). Appropriate amount of enzyme (100 µL) was added to 1mL

of Bradford reagent and the intensity of color developed was checked spectrophotometrically at 595 nm.

Kinetics of CMCase Production

CMCase was produced by *H. insolens* under submerged conditions. Effect of temperature, pH, substrate concentration and inoculums density on the kinetics of CMCase production was determined varying one parameter at a time (Javed *et al.*, 2011; Huma *et al.*, 2012).

Effect of Inocula Density

Different inocula sizes: 4, 6, 8, 10 and 12% (v/v) were used to study the effect on growth kinetic parameters for CMCase production. Study was carried out at 45°C, pH 5.0, substrate (CMC: 2% w/v) in orbital shaker at 100 rpm revolution for 90 h. The optimum inoculum density was found to be 8% w/v, which was fixed for the subsequent experiments.

Effect of Substrate

Effect of substrate (CMC) concentrations (1–10% w/v) on the enzyme production was studied under submerged conditions at pH 5.0 and 45°C. The inoculum density used was 8% (v/v). Various time course aliquots were taken and checked for extracellular protein, CMCase activity and biomass content. The optimum substrate concentration was 5% was used in further experiments (Javed *et al.*, 2011; Huma *et al.*, 2012).

Effect of pH

The optimum pH for *H. insolens* growth was determined by cultivating the fungus at 45°C in growth media having varied pH (4.0 – 6.0) with an increment of 0.5. The inoculums density and CMC concentration was 8% (v/v) and 5% (w/v), respectively. The pH of Eign and Push medium was adjusted with 1M HCl/1M NaOH. Time course aliquots were withdrawn aseptically after appropriate time intervals and assayed for extracellular protein, enzyme activity and cell mass.

Effect of Temperature

To evaluate thermodynamic parameters and optimum temperature for the production of CMCase by *H. insolens*, the fungus was grown at temperatures 36, 39, 42 and 45°C. The other growth conditions were as follows: CMC= 5%; pH=5.0; inoculums density= 8%. Time course aliquots were taken and analyzed as mentioned in previous sections.

Determination of Growth Kinetic Parameters

Growth kinetic parameters for *H. insolens* were determined as described by Javed *et al.* (2011) and Huma *et al.* (2012).

Specific growth rate (μ) was calculated from slope of plot: ln x vs Time –1.

Biomass (cell mass) doubling time (t_d) = ln 2/μ –2.

Product yield coefficient with respect to cell mass=$Y_{p/x}$ –3.

Where, p = CMCase units and x = cell mass at the time of maximum CMCase production.

Specific rate of product formation (q_p) = $Y_{p/x} \times \mu$ –4.

Maximum specific rate of cell mass formation (μ_m) was determined from a double reciprocal plot: 1/μ vs 1/s (substrate), which was equal to intercept on ordinate (1/μ_m). Similarly maximum rate of CMCase production (q_{pmax}) was determined from the plot: 1/q_p vs. 1/s.

Activation energy for cell mass [$Ea_{(x)}$] and CMCase production [$Ea_{(p)}$] were determined by applying Arrhenius plots: (ln μ vs. 1/T) and (ln q_p vs. 1/T), respectively.

Determination of Thermodynamics of CMCase Production

Thermodynamic parameters for cell mass and the enzyme production by *H. insolens* were calculated by using the rearranged Eyring's absolute rate equation derived from the transition state theory.

$$k = (k_b.\ T/h).e^{(-\Delta H^*/RT)}.e^{(\Delta S^*/R)} \quad –5.$$

Where: k_b: Boltzmann's constant = 1.38×10^{-23} JK^{-1}; T = Absolute temperature (K); h is Planck's constant = 6.626×10^{-34}Js; N is Avogadro's number = 6.02×10^{23} mol^{-1}; R: Gas constant = 8.314 J $K^{-1}mol^{-1}$; ΔH^* and ΔS^* are change in Enthalpy and Entropy for activation of product formation, respectively.

$\Delta H^* = Ea\text{-}RT$ –6.

ΔG^* (Change in Gibbs free energy) = $-RT \ln (k.h/k_b.T)$ –7.

$\Delta S^* = (\Delta H^* - \Delta G^*)/T$ –8.

The μ_m and q_{pmax} are equivalent to k (Monod, 1942). ΔH^*, ΔG^* and ΔS^* were calculated by applying Equations 6–8 with the modification that in Eq.–7 k was replaced with μ_m for cell mass formation and q_{pmax} for CMCase production. Moreover, in Eq.–6 Ea was replaced by $Ea_{(x)}$ and $Ea_{(p)}$ for the cell mass and product (CMCase) production, respectively.

Results

Kinetics of CMCase Production by *Humicola insolens*

The effect of physiochemical factors on the productivity of endoglucanases by a local thermophillic fungal strain of *H. insolens* was studied. The kinetic and thermodynamic characterization of CMCase produced was also carried out.

Effect of Inoculum

CMCase was produced by *H. insolens* on 2% CMC at 45°C and pH 5.0 using various inoculum densities [4%, 6%, 8%, 10% and 12 (% v/v)]. Maximum CMCase production i.e., 0.79 ± 0.04 was observed at 8% (12.41 mg cells mL^{-1})

inoculum size. Total protein and specific activity at the time of maximum CMCase production were 0.11±0.006 and 7.4, respectively. The specific growth rate (μ) and t_d for 12.41 mg mL^{-1} cells were 0.06 and 11.8, respectively. The t_d was lowest among all inoculum sizes, while $Y_{p/x}$ and q_p (specific rate of product formation) at12.41 mg mL^{-1} cells were maximum i.e., 0.0047 and 0.000278, respectively (Table 1).

Effect of Substrate

CMCase was produced by *H. insolens* on varying concentrations of carbon source i.e. CMC at 45°C and pH 5.0 using optimized 8% (12.41 mg cells mL^{-1}) inoculum size. 5% CMC showed maximum CMCase production i.e. 2.0± 0.110 and total protein at that time was 0.131±0.007, while specific activity was 15.45. The μ and t_d were 0.0461 and 15.01, respectively. $Y_{p/x}$ and q_p at optimum substrate concentration were 0.0139 and 0.000643, respectively (Table 2).

Effect of pH

Effect of pH on CMCase production by *H. insolens* was observed at pH: 4.0, 4.5, 5.0, 5.5 and 6.0 at 45°C, keeping all the pre-optimized conditions same. Highest CMCase production was 2.0±0.1 U mL^{-1} at pH 5.0 and the extracellular protein at the time of maximum CMCase production was 0.13 mg mL^{-1}, while specific activity was 15.45. The μ and t_d were 0.046 and 15.0, respectively. $Y_{p/x}$ and q_p at optimum pH were 0.0139 and 0.00064, respectively (Table 3).

Effect of Temperature

The *H. insolens* was grown at various temperatures i.e., 36°C, 39°C, 42°C and 45°C for the optimal production CMCase using pre optimized conditions. Maximum CMCase production i.e. 1.99±0.121 was observed at 45°C. Total protein at the time of maximum CMCase production was 0.129±0.007, while specific activity was 15.42. The μ and t_d were found to be 0.046 and 15.02, respectively. $Y_{p/x}$ and q_p were 0.0139 and 0.00064, respectively at 45°C (Table 4).

Thermodynamics of CMCase Production

Arrhenius plot was applied to determine the activation energy (E_a) for cell mass formation. The slope value from the plot: μ Vs 1/T was positive, hence $E_{a(m)}$ for cell mass formation could not be determined (Fig. 2). Moreover, the double reciprocal plot between 1/s and 1/μ could not be applied to determine the μ_{max} because the graph line did not intersect the –X-axis line.

Activation energy for product formation ($E_{a(p)}$) i.e. CMCase production was calculated by applying Arhenious plot and was 108.69 KJ $mole^{-1}$ (Fig. 3). The $q_{p(max)}$ was obtained from Fig. 4 and was equal to 0.0011 U mg^{-1} cell mass. Change in enthalpy (ΔH^*_p), Gibbs free energy, (ΔG^*_p) and entropy (ΔS^*_p) for CMCase production were 106.05 KJ $mole^{-1}$, 117.59 KJ $mole^{-1}$ and -36.30 $J.mole^{-1}K^{-1}$, respectively.

Discussion

Increased interest in cellulases is because of its wide range on industrial applications including textile and paper industry, animal food and bioethanol production etc., (Jabbar *et al*., 2008; State *et al*., 2010). Cellulases have been produced by different microbes including bacteria, protozoans and many filamentous fungi like *T. ressei, T. viride, A. niger, A. citrinus, G. citrine, Humicola* species (Jabbar *et al*., 2004, 2008; Leite *et al.*, 2008).

It is necessary to determine optimum inoculum size for the optimum enzyme production. Small inoculum level may give low cell mass, therefore, decreasing product formation while higher inoculum density utilizes nutrients quickly, giving large amount of cell mass resulting in less product formation (Sharma *et al*., 2008). Results showed that optimum inoculum size for CMCase production was 8% (12.41 mg cells mL^{-1}). Further increase in inoculum decreased the enzyme production. Javed *et al*. (2011) reported that optimum inoculum size for β-glucosidase production from mutant derivative of *A. niger* was 10% v/v. In another report, Huma *et al*. (2012) has also found optimum inoculum level of 10% (v/v) for α-amylase production by *Phialocephala humicola*.

Among various concentrations of CMC i.e., (1, 2, 3, 4, 5, 6, 8 and 10%) investigated, the maximum CMCase production was obtained at 5%, which decreased with increase in substrate concentration. It may be because of the reason that increase in substrate concentration decreases the porosity and aeration of medium, which are necessary for appropriate growth of organism (Bigelow and Wyman, 2002). The values of $Y_{p/x}$, q_p and specific activity were maximum at 5% which showed decreasing trend with increase in substrate concentration. Raza and Rehman (2009) studied the effect of substrate on endo-β-1,4-glucanase production by a thermophillic fungus. Results revealed that CMCase production was high for wheat straw i.e., 5.0 U mL^{-1} as compared to wheat straw and wheat bran i.e., 2.50 U mL^{-1} and 1.62 U mL^{-1}, respectively. Optimum substrate concentration for CMCase production by *A. flavus* was 4% wheat bran. Wheat bran proved to be the best substrate for the production of CMCase (Gomathi *et al*., 2012).

The pH of the growth medium plays a very important role in regulating the production of enzymes and growth rate of microbes. *H. insolens* showed optimum production of CMCase at pH 5.0. Effect of culture medium pH on kinetics of CMCase production was studied by using CMC (5%) at 45ºC. The results indicated that optimal kinetic parameters of *H. insolens* were achieved in pH range of 4.5-5.5, while highest CMCase production was attained at pH 5.0. Further increase or decrease in pH resulted in decrease of CMCase

Table 1: Effect of Inoculum size on the kinetics of CMCase by *H. insolens* grown on CMC under submerged conditions

Inoculum density	CMCase (U mL^{-1})	TP (mg mL^{-1})	μ (h^{-1})	t_d (h)	$Y_{p/x}$ (U mg^{-1})	q_p (U mg^{-1} h^{-1})	Sp. Activity (U mg^{-1})
4%	0.21 ± 0.01	0.07 ± 0.004	0.06	11.9	0.0013	0.000076	3.1
6%	0.45 ± 0.02	0.09 ± 0.005	0.06	12.0	0.0028	0.000159	4.8
8%	0.79 ± 0.04	0.11 ± 0.006	0.06	11.8	0.0047	0.000278	7.4
10%	0.32 ± 0.02	0.05 ± 0.002	0.05	14.9	0.0021	0.000097	6.9
12%	0.30 ± 0.02	0.04 ± 0.002	0.05	15.0	0.0019	0.000086	6.8

Table 2: Effect of substrate concentration on kinetics of CMCase by *H. insolens* grown on CMC under submerged conditions

Substrate conc. (w/v)	CMCase (U mL^{-1})	TP (mg mL^{-1})	μ (h^{-1})	t_d (h)	$Y_{p/x}$ (U mg^{-1})	q_p (U mg^{-1} h^{-1})	Sp. Activity (U mg^{-1})
2%	0.79 ± 0.04	0.10 ± 0.006	0.060	11.8	0.0047	0.000278	7.4
3%	0.84± 0.042	0.104±0.003	0.0468	14.79	0.0062	0.000292	12.09
4%	1.01± 0.058	0.110±0.005	0.0475	14.57	0.0077	0.000368	12.94
5%	2.0± 0.110	0.131±0.007	0.0461	15.01	0.0139	0.000643	15.45
6%	1.72± 0.099	0.123±0.006	0.0460	15.06	0.0112	0.000516	14.22
8%	1.65± 0.095	0.121±0.006	0.0459	15.08	0.0104	0.000481	13.94
10%	1.64± 0.095	0.120±0.006	0.0458	15.12	0.01018	0.000466	13.65

Table 3: Effect of pH on the kinetics of CMCase by *H. insolens* grown on CMC under submerged conditions

pH	CMCase (U mL^{-1})	TP (mg mL^{-1})	μ (h^{-1})	t_d (h)	$Y_{p/x}$ (U mg^{-1})	q_p (U mg^{-1} h^{-1})	Sp. Activity (U mg^{-1})
4.0	1.7 ± 0.1	0.09 ± 0.005	0.057	12.2	0.0133	0.00076	19.42
4.5	1.9 ± 0.1	0.12 ± 0.007	0.051	13.5	0.0139	0.00071	15.78
5.0	2.0 ± 0.1	0.13 ± 0.007	0.046	15.0	0.0139	0.00064	15.45
5.5	1.9 ± 0.1	0.04 ± 0.002	0.052	13.4	0.0138	0.00072	52.83
6.0	1.7 ± 0.1	0.03 ± 0.002	0.054	12.8	0.0131	0.00071	64.71

Table 4: Effect of temperature on CMCases production by *H. insolens* grown on CMC (5% w/v) and pH 5.0 in submerged conditions

Temp (°C)	CMCase (U mL^{-1})	TP (mg mL^{-1})	μ (h^{-1})	t_d (h)	$Y_{p/x}$ (U mg^{-1})	q_p (U mg^{-1} h^{-1})	Sp. Activity (U mg^{-1})
36	0.45±0.03	0.033±0.001	0.057	12.13	0.0033	0.000188	13.63
39	0.71±0.04	0.039±0.002	0.054	12.91	0.0052	0.000279	18.20
42	1.19±0.03	0.085±0.004	0.049	14.01	0.0084	0.000411	14.00
45	1.99±0.12	0.129±0.007	0.046	15.02	0.0139	0.00064	15.42

Data presented are average values ± SD of n = 3 experiments. μ = specific growth rate, t_d = biomass doubling time, $Y_{p/x}$ = product yield coefficient with respect to cell mass, q_p = specific rate of product formation

production because organism may need acidic pH to some extent for its growth and enzyme production as metabolic activities of microbes and their growth are very sensitive to change in pH (Gomathi *et al*., 2012). Sherief *et al*. (2010) has also reported optimum pH 5-6 for maximum CMCase production.

Temperature is a very critical parameter and sometimes a slight change in it may considerably affect the enzyme production by the microbes. The growth of *H. insolens* on carboxymethyl cellulose was strongly affected by temperature and the strain showed a reasonable amount of CMCase production at a temperature range of 36-45°C. At higher temperatures (above 45°C) the enzyme secretion was very low, while the optimum temperature for CMCase biosynthesis was 45°C. The maximum CMCase production, maximum specific growth rate and the maximum product formation rate was at 45°C and decreased with increase in temperature because increase in temperature may inhibit fungal growth or the high temperature of medium may change membrane composition. According to Raza and Rehman (2009) the optimum temperature on endo-ß-1,4-glucanase production by a thermophillic fungus was 60°C.

In conclusion, a number of microbial strains exist for efficient production of CMCase, but only a few fungi meet the criteria for commercial production. *H. insolens* has potential for industrial applications.

Acknowledgement

Results presented in this paper are part of PhD thesis of Hina Riaz. The research work was partially sponsored by HEC, Islamabad under the Indigenous Ph.D. Scholarship scheme and PAEC, Pakistan. Technical services of Ghulam Ali Waseer are highly valued.

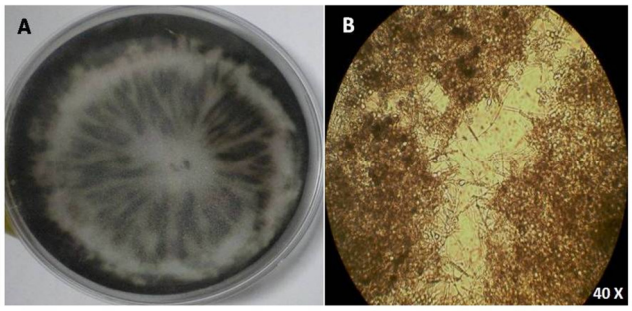

Fig. 1: A: Growth of *H. insolens* on PDA plate. **B**: Microscopic view of the fungus.

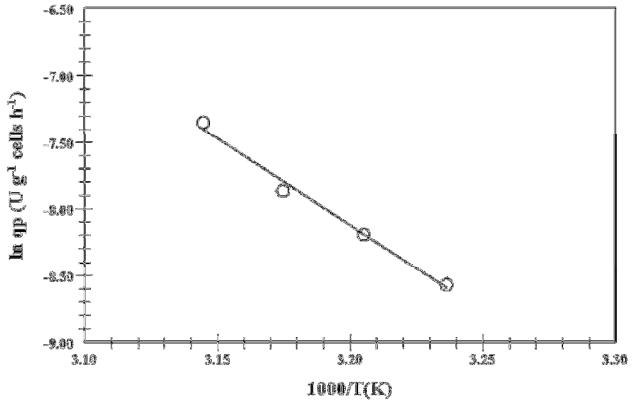

Fig. 2: Arrhenius plot for the determination of activation energy for CMCase production by *H. insolens*

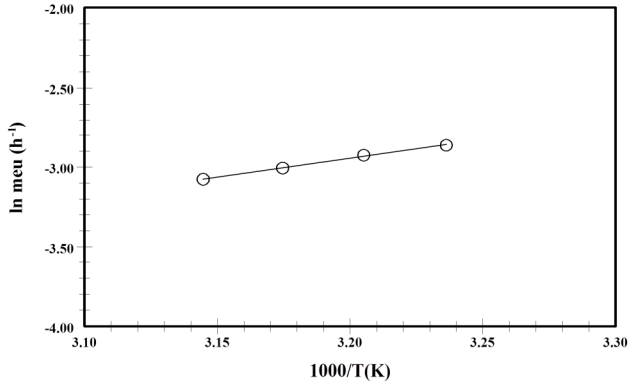

Fig. 3: Arrhenius plot for the determination of activation energy for cell mass formation of *H. insolens*

References

Bhatti, H.N., S. Batool and N. Afzal, 2013. Production and characterization of a novel β-glucosidase from *Fusarium solani. Int. J. Agric. Biol.*, 15: 140–144

Javed, M.R., M.H. Rashid, Z. Mukhtar, M. Riaz, H. Nadeem, T. Huma and N. Ashiq, 2011. Kinetics and thermodynamics of high level β-glucosidase production by mutant derivative of *Aspergillus niger* under submerged growth conditions. *Afr. J. Microbiol. Res.*, 5: 2528–2538

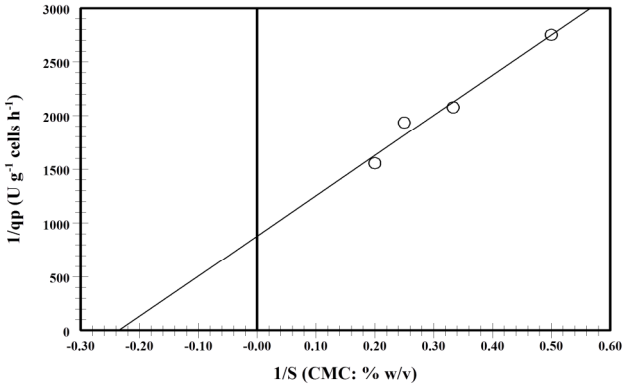

Fig. 4: Double reciprocal plot for the determination of maximum specific rate of CMCase production [$q_{p(max)}$] by *H. insolens*

Khan, J.A. and S.K. Singh, 2011. Production of cellulase using cheap substrates by solid state fermentation. *Int. J. Plant Anim. Environ. Sci.*, 1: 179–187

Leite, R.S.R., 2008. Production and characteristics comparison of crude ß-glucosidases produced by microorganisms *Thermoascus aurantiacus* and *Aureobasidium pullulans* in agriculture wastes. *Enzyme Microb. Technol.*, 43: 391–395

Luqman, A., T. Rizwan, M.I. Rajoka and F. Latif, 2010. Cloning and expression of avicelase gene of *Humicola insolens* in *Pichia pastoris*. *J. Biotechnol*., 150: 155–156

Lynd, L.R., P.J. Weimer, W.H. Van Zyl and I.S. Pretorius, 2002. Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiol. Mol. Biol.,* 66: 506–577

Nadeem, H., M.H. Rashid, M. Riaz, B. Asma, M.R. Javed and R. Perveen, 2009. Invertase from hyper producer strain of *Aspergillus niger*: Physiochemical properties, thermodynamics and active site residues heat of ionization. *Protein Pept. Lett*., 16: 1098–1105

Niaz, M., T. Iftikhar and M.H. Rashid, 2011. Carboxyl group modification of *Gymnoascella citrina* glucoamylase: Cross-linking with hydrophobic nucleophile enhanced thermostability and thermophilicity. *Clin. Biochem.*, 44: S93–S94

Pirt, S.J., 1975. *Principles of Microbe and Cell Cultivation*, 2nd edition, Vol. 99, pp: 116–124. London, Blackwell Scientific

Raza, M.A. and S.U. Rehman, 2009. Production and characterization of endo-ß-1,4-glucanase from *thermophilic fungus. Afr. J. Biotechnol.*, 8: 3297–3302

Reddy, S.S., E.Z. Nyakatawa and K.C. Reddy, 2012. Nitrogen uptake pattern by cotton in a long-term no-tillage system with poultry litter application. *Int. J. Agric. Biol*., 14: 29–37

Riaz, M., M.H. Rashid, L. Sawyer, S. Akhtar, M.R. Javed, H. Nadeem, and M. Wear, 2012. Physiochemical properties and kinetics of glucoamylase produced from deoxy-D-glucose resistant mutant of *Aspergillus niger* for soluble starch hydrolysis. *Food Chem.,* 130: 24–30

Sharma, A., V. Vivekanand and R.P. Singh, 2008. Solid-state fermentation for gluconic acid production from sugarcane molasses by *Aspergillus niger* ARNU-4 employing tea waste as the novel solid support. *Bioresour. Technol*., 99: 3444–3450

Sherief, A.A., A.B. El-Tanash and N. Atia, 2010. Cellulase production by *Aspergillus fumigatus* grown on mixed substrate of rice straw and wheat bran. *Res. J. Microbiol*., 5: 199–211

State, M.A.M., A. Hammad, M. Swelim and R.B. Gannam, 2010. Enhanced production of cellulases by *Aspergillus* spp. isolated from agriculture wastes by solid state fermentation. *A.E. J. Agric. Environ. Sci*., 8: 402–410

Walsh, G., 2002. Industrial enzymes: proteases and carbohydrases. In: *Proteins, Biochemistry and Biotechnology*. John Wiley and Sons, Ltd., New York, USA

(Received 16 May 2013; Accepted 30 July 2013)