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RESEARCH ARTICLE

STUDY ON DIFFERENT PARAMETERS DURING HYDROLYSIS OF COLLOIDAL CHITIN USING *TRICHODERMA HARZIANUM*, A VEGETATIVE FUNGUS

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ABSTRACT

Chitin is the only nitrogen based abundantly available natural biopolymer and its derivatives have wide applications in food/health supplements, cosmetics, biomaterials, coagulants and adsorbents used in water treatment. Species of *Trichoderma* are effective chitinolytic enzyme producing fungi. The raw chitin procured from Enviro Biotech Pvt. Ltd, is used as substrate for enzymatic degradation by *Trichoderma* species, which acts only on modified chitin, thus the raw chitin is treated with mineral acid to obtain colloidal form. Hydrolysis experiments are carried out under optimum conditions of media pH and temperature of incubation to study the production of N- acetyl D- glucosamine (GlcNAc) and tyrosine. Seed media, chitinolytic enzyme concentrate, inoculum containing varying pellet numbers were utilized as source of enzyme. Experiments are conducted as function of incubation time, volume and pH of enzyme concentrate, substrate concentration and speed of orbital shaker. Oxygen transfer phenomenon is studied by means of volumetric oxygen transfer coefficient using lab scale fermenter to understand the hydrolysis of colloidal chitin in different environment but under same optimum conditions.

INTRODUCTION

Chitin, a 1, 4-β- linked polymer of N- acetyl D- glucosamine, is the most abundant natural amino polysaccharide and is estimated to be produced annually almost as much as cellulose. Chitin is found in the exoskeleton as well as in the internal structures of invertebrates and it is a white, hard, in-elastic, nitrogenous polysaccharide. The enzymatic degradation of chitin by microorganisms occurs in two consecutive steps; first, the hydrolysis by chitinase to oligomers, mainly dimers and the next step is followed by their degradation to free N-acetyl D- glucosamine by chitobiase. The monosaccharide released can then be metabolized by many microorganisms (Pradip Kumar Dutta *et al.,* 2004). These enzymes are widely distributed in nature and have been found in most of the bacteria and fungi (Cirano *et al.,* 1991). The release of spores and stipe elongation in some basidiomycetes (Majeti and Ravi Kumar, 2000; Michele Michelin *et al.*, 2013) and autolysis of mycelium in cultures are all functions of fungal chitinases (Rodriguez Porcel *et al.,* 2005; Kapat *et al.,* 1996). In recent years, the several works have suggested that the chitinase-producing fungi, e.g. species of *Trichoderma,* are most effective biological control agents against fungal pathogens (OlaGomaa and Heba El Bialy, 2009; Syahiddin Dahlan Said, 2007). Many of literatures have most commonly focused on the effects of mechanical forces on fungal morphology and mycelial cultures consisting of clumps and freely dispersed hyphae. In general, the little knowledge is about the influence of shear stresses on pelleted fungal cultures. It is easy to mix and aerate the broths in pelleted growth because of its less viscosity in comparison with filamentous growth. The relationship between the pellet morphology, as influenced by agitation intensity, and the bulk rheology of broths of the filamentous micro fungus *Aspergillus terreus*. In a conventional stirred tank fermenter and also a fluidized bed bioreactor these effects were examined. In both systems, to separate the influence of agitation from the oxygen transfer effects the dissolved oxygen concentration was held constant at 400% of air saturation. The stirred tank reactor was operated at 300, 600 and 800 rpm and the fluidized bed reactor was aerated at 1 vvm. The pellet morphology and bulk broth rheology were measured for various agitation conditions in the bioreactors (Acharya and Katyare, 2004; Jayakumar *et al.,* 2011).

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MATERIALS AND METHODS

Organism used: *Trichoderma harzianum* MTCC 3928 obtained from the Institute of Microbial Technology,

Chandigarh, India is used for chitin hydrolysis. The organism is maintained on MRBA (Medium Rose Bengal Agar) plates. 31.55 g/L of Rose Bengal Agar Base (M842) in distilled water is prepared and used as nutrient media for pour plate culture development. Liquid media is poured onto sterile petri plates under aseptic conditions. The plates are inoculated with culture; the inoculated plates are incubated at 32^{o}C ±2^{o}C for 105 h. Culture plate is stored at 4^{o}C±2 ^{0}C to arrest the growth of culture and used as inoculum for further sub culturing.

Preparation of growth medium for T. harzianum (seed)

Growth medium of T**.** *harzianum* is prepared as given in table. Solution pH is adjusted to 5 using 1N NaOH. The media is sterilized by autoclaving at 15 lb/in^2 pressure (121^{o}C) for 20 min. It is cooled to room temperature. 10 mL of the media is transferred (inside a laminar flow chamber) in to a sterile 20 mL test tube. Media containing test tube is inoculated from a fresh 105 h working pour plate above the flame in the laminar flow chamber. Cell suspension is mixed well using vortex mixer. Cell suspension is measured for *c*ell concentration. 100 mL of seed growth medium is inoculated with one milliliter of cell suspension containing T. *harzianum*. Inoculated media is incubated on orbital shaker maintained at 32^{o}C for 43 h to grow pellets which is used as inoculum for chitin hydrolysis.

Table 1. Growth (seed) medium for T.*harzianum*

Constituents	Concentration, g/L
Glucose	10
$(NH_4)_2SO_4$	1.4
KH_2PO_4	2.0
$NaH_2PO_42H_2O$	6.9
$MgSO_47H_2O$	0.3
Peptone	1.0
Citric acid monohydrate	10.5

Inoculum formulation for hydrolysis using different pellet concentration (number)

100 mL of seed media developed in triplicates is used to prepare inoculum with variable pellet number. 10 mL of seed media solution is considered in triplicate to count the pellet number from each of the seed media developed. The average of the count is used as pellet concentration. Pellets (5, 10, and 15) are used as inoculum for hydrolysis.

Substrate modification and medium formulation

The raw chitin is modified to activate the surface area for enzymatic reaction. The details of the pretreatment, the proportion of the nutrient media and formulation of enzyme production media and preparation of hydrolysis media are discussed in this section.

Pretreatment of the raw chitin for colloidal chitin Preparation

12.5 g of raw chitin was treated with 100 mL of concentrated (12N) HCl, a mineral acid. The suspension was mixed well for about 5 h. Gelatinous paste obtained is stored at 4 in the refrigerator for about 24 h. The brown color solution resulted from dissolution of chitin was added with 1 L of water, agitated for 10 h and allowed to settle. The supernatant solution was discarded and later treated with 1 L of 1N NaOH solution for neutralization. The suspension is centrifuged to remove dissolved proteins (supernatant) at 10000 rpm for 10 min and supernatant is analyzed for protein content. The suspended colloidal chitin is used as substrate for hydrolysis by *Trichoderma harzianum.*

Formulation of enzyme production medium

The enzyme production medium is prepared as per the composition given in Table 2 and used as source of enzymes for hydrolysis. The constituents are dissolved in distilled water to prepare 1 L of enzyme production media. The pH of the media is adjusted to 6 using 2N NaOH. The media is sterilized at 15 lb/in^2 (gauge) pressure (121^{o}C) for 20 min. Sterile media is cooled to room temperature and preserved at 4 for hydrolysis.

Table 2. Composition of enzyme production medium

Constituents	Concentration,g/L
Chitin	5
$(NH_4)_2SO_4$	1.4
KH_2PO_4	2.0
$NaH_2PO_4.2H_2O$	6.9
$MgSO_4.7H_2O$	0.3
Urea	0.3
peptone	1.0
Tween 80	0.2
$FeSO_4.7H_2O$	0.005
$MnSO_4$	0.0016
$ZnSO_4$.$7H_2O$	0.0014
$CaCl_2.2H_2O$	0.002

Medium formulation for chitin hydrolysis (Patil *et al.* 1999)

The hydrolysis media is prepared using the concentration of nutrients and substrate as in the Table 3. The constituents are dissolved in distilled water and pH of the media is adjusted to 5. Conical flasks containing media are sterilized at 121 at 15 lb/in^2 (gauge) pressure for 20 min. After sterilization the media is allowed to cool to room temperature to make the temperature feasible for inoculation.

Table 3. Hydrolysis media composition for enzymatic conversion

Constituents	Concentration,g/L
Chitin	12.5
(NH4)2SO4	4.2
KH2PO4	2.0
NaH2PO4.2H2O	6.9
MgSO4.7H2O	0.3
Tween 80	0.2
FeSO4.7H2O	0.005
MnSO4	0.0016
ZnSO4.7H2O	0.0014
CaCl2.2H2O	0.002

Estimation of N-acetyl glucosamine by Di Nitro Salicylic acid (DNS) method (Miller, 1959)

0.5 mL of culture filtrate sample is taken in amber tube and volume is made to 1 mL by adding 0.5 mL of distilled water. Blank solution is prepared in amber tube with 1 mL distilled water. 1 mL of DNS is added to each of the amber tubes and is closed. Reaction mixture is heated for 10 min in boiling water for completion of the reaction between reducing sugar

(glucose) and DNS. Reaction mixture is cooled to 40 and & 1 mL sodium potassium tartrate (SPT) is added. SPT is used to stabilize the reaction. Reaction mixture is cooled to 30 ^{o}C and total volume is made to 10 mL using distilled water. Absorbance is read at 540 nm using spectrophotometer.

Enzyme assay (Subramanya and Rao, 1987)

Purpose of performing enzyme assay is to find out the enzyme activity in unit (U). One unit (U) of enzyme activity is defined as the amount of enzyme required for the formation of 1 μmol of the product per minute of the reaction under the standard assay conditions. Acid swollen chitin required for enzyme assay is prepared as explained below. 1 g of colloidal chitin is added to 10 mL of 85% orthophosphoric acid. The mixture is stirred well and re-suspended into excess of cold (2-4 mL at 15^{o}C) distilled water, then resuspended in 200 mL of sodium acetate buffer (50 mM, pH4.75) (Subramanya and Rao, 1987; Mako *et al.,* 2006; Rodde *et al.* 2008). Suspension is stored at 10^{o}C for further use up to a maximum of one month. Toluene is added as a preservative at the concentration of 1% (volume).

Estimation of Tyrosine (Nickos *et al.,* 1993)

0.5 mL of culture filtrate is taken in an amber tube and the volume is made to 1 mL by adding 0.5 mL of distilled water. Blank solution is prepared from 1 mL of distilled water. 5 mL of RC reagent into each tubes and the mixture is incubated at 30 for 10 min. 0.5 mL of Folin-Ciocalteau reagent is added and mixed thoroughly. The reaction mixture is incubated for 30 min at 30 and the absorbance is measured at 720 nm using spectrophotometer.

Hydrolysis with enzyme concentrates of pH 5 and 6

The enzyme production medium is prepared as per the composition given in Table 2 and used as source of enzymes for hydrolysis. Experiments are conducted in triplicates at two different sets of pH 5 and 6. The hydrolysis media is prepared. The composition of media is given in Table 3. The constituents are dissolved in distilled water and pH of the media is adjusted to 5 and 6 using 2M NaOH solution. Later the media is distributed into conical flasks (100 mL each) and covered with cotton plugs. Conical flasks containing media are sterilized, the culture filtrate (enzyme solution of pH5 and 6) is used as inoculum and the experiment is carried out in duplicates and the concentration of inoculum is varied as 1, 3, 5, 10, 15, 20, and 25 mL. The inoculated conical flasks are kept in shaking incubator at 32(+/-2) C at 180 rpm and experiment is carried for six days and samples are collected after every 24 h. Samples are analyzed for the GlcNAc concentration and protein released during hydrolysis. Results are compared to find out volume of inoculum which gives maximum yield.

Substrate concentration dependency on hydrolysis of chitin

As colloidal chitin is the main substrate for chitin hydrolysis, the experiments are performed to find optimum substrate concentration. 1 L of hydrolysis media is prepared using distilled water as per the concentration given in Table 3.Experiments are carried out in triplicates. The substrate for chitin hydrolysis is added to the hydrolysis media by varying concentration like 1 g, 2 g and 3 g. The media is distributed into conical flasks and pH of the media is set to 5 and covered with cotton plug and sterilized at 121 for 20 min at 15 lb/in^2 (gauge) pressure. Media is cooled to room temperature and inoculated with seed media of 43 h culture in laminar air flow chamber. Hydrolysis is carried for six days in orbital shaker at 32 at 180 rpm. Samples were collected and analyzed for GlcNAc concentration and protein concentration to compare the yields chitin hydrolysis of varied substrate concentration.

Effect of pellet concentration (number) on hydrolysis of colloidal chitin at different agitation speed

The hydrolysis media was prepared as per the procedure and pH is set to 5 and sterilized to make sure of aseptic conditions for inoculation. 43 h old pellets were counted and inoculated in the hydrolysis media and flasks are kept in orbital shaker for six days at 32 at 180, 130 and 210 rpm. Hydrolysis is carried out with different number of pellets like 5, 10 and 15 and experiments were carried out in triplicates. Samples are collected after every 24 h and analyzed for GlcNAc concentration released during hydrolysis.

Determination of mass transfer coefficient during hydrolysis of colloidal chitin in laboratory scale fermenter

Mass transfer coefficient is determined by "Dynamic gassing out" method in the bioreactor during hydrolysis of colloidal chitin. The laboratory scale stirred fermenter is almost similar to bioreactors used in industries for large scale production. This fermenter is assembled with different probes to regulate conditions such as pH, dissolved oxygen (DO) and temperature. 3 L of hydrolysis media is prepared according to the Table 3. Colloidal chitin is added into the reactor and pH of the media is set to 5 using 4M NaOH. All knobs of reactor are closed with cotton plug and are kept for autoclaving to sterilize the media containing colloidal chitin. The media is sterilized; 30 mL of 43 h old culture containing vegetative fungi is used as inoculum. Reactor containing media is inoculated aseptically inside the laminar air flow chamber. The reactor is assembled and pH of the reaction media is maintained throughout hydrolysis using 4N HCl and NaOH solutions.

Figure 2. Photograph of lab fermenter with digital display to monitor the bioprocess

The reaction is carried out for four days and DO level (C_0) is noted down. On fifth day, the media is sparged with nitrogen gas to reduce DO level. Purging with nitrogen is continued until DO level falls close to zero. Media is sparged with air to

increase the DO level which is monitored and recorded with the help of DO probe and the software connected to the system. Experiment is continued till the oxygen level reaches to its saturation(C*) and is noted down. Further the volumetric oxygen transfer coefficient is calculated by plotting the graph of ln (C*-C) versus time.

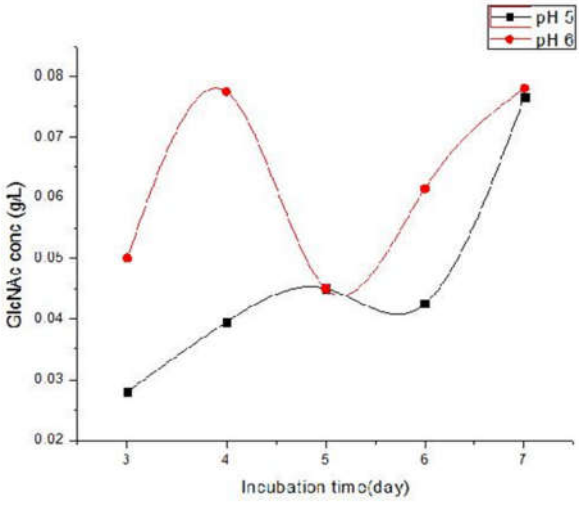
RESULTS AND DISCUSSION

GlcNAc concentration, tyrosine and enzyme assay using enzyme production media of pH 5 and 6

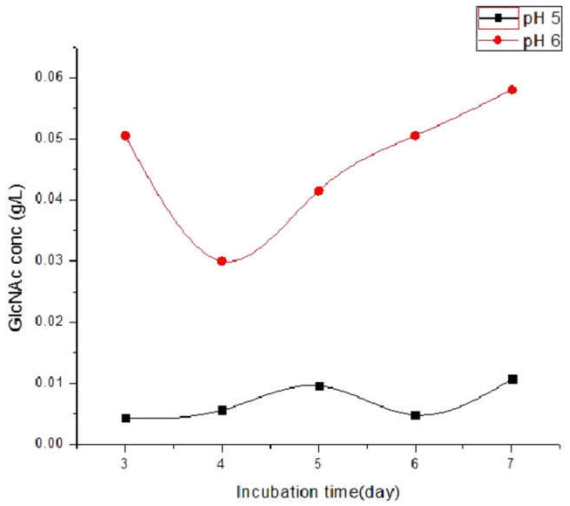
The hydrolysis of enzyme production media to obtain enzyme solution is carried out for seven days in orbital shaker and the experiments are carried out with two different pH values such as 5 and 6. Collected samples are analyzed for production of GlcNAc, enzyme activity and proteins released during hydrolysis of colloidal chitin. The amount of GlcNAc concentration produced during hydrolysis varies with value of varied pH of hydrolysis media. By studying both experiments, it is clear that the maximum GlcNAc is obtained on seventh day at pH 6 when compared to that of hydrolysis of colloidal chitin at pH 5 and it is shown in the following graph1; Enzyme activity is measured by the amount of GlcNAc released by the enzymes at different intervals of time during hydrolysis of colloidal chitin and it is observed that maximum result is obtained on the seventh day of the experiment with hydrolysis media of pH 6 where the enzyme activity is comparatively higher than that of enzyme produced in hydrolysis media of pH 5. And it is shown in following graph 2; the amount of protein released during hydrolysis of enzyme production media containing colloidal chitin is increasing with respect to time. Maximum proteins content is obtained on seventh day of both the experiment of hydrolysis media of pH 5 and pH 6 and it is represented in below graph 3.

Effect of enzyme concentrate at different pH 5 and 6 on hydrolysis of colloidal chitin

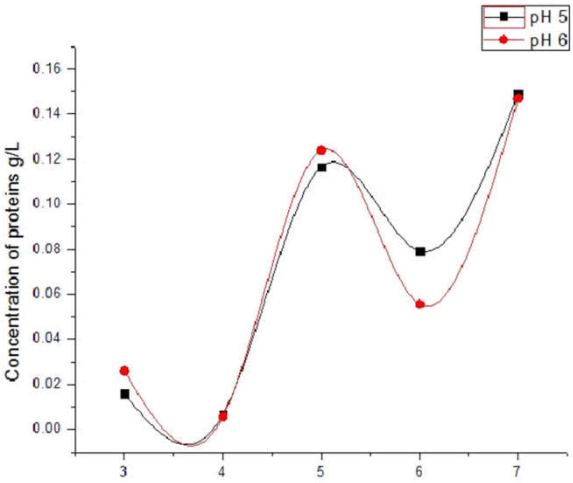
This is to compare the results of hydrolysis media inoculated with culture filtrate of enzyme solution of pH 5 and 6 by varying the concentration of inoculum such as 1 mL, 3 mL, 5 mL, 10 mL and 15 mL.



Graph 1. GlcNAc concentration (g/L) produced with enzyme solution of pH 5 and pH 6

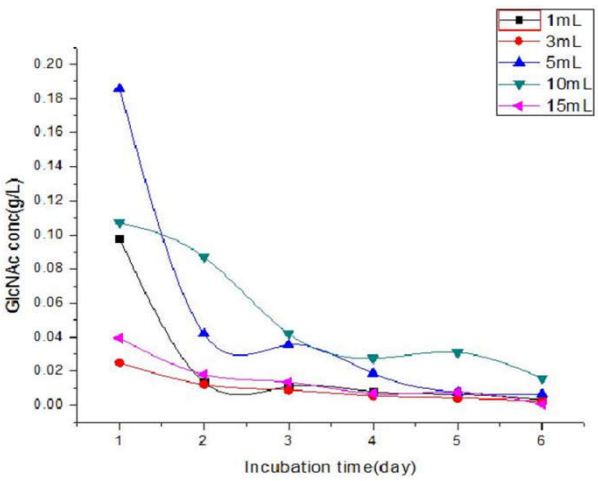


Graph 2. Tyrosine concentration (g/L) produced with enzyme solution of pH 5 and pH 6

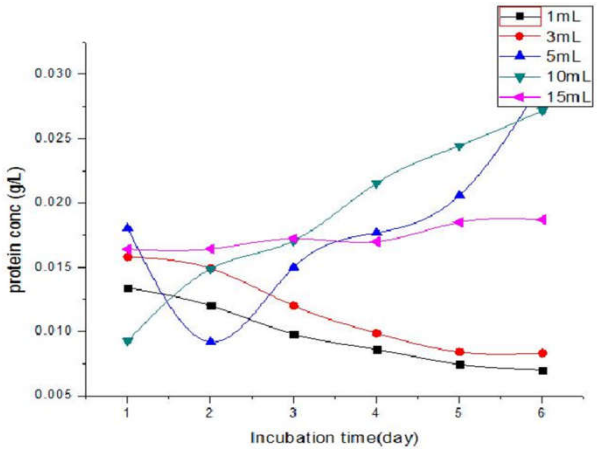


Graph 3. Concentration (g/L) analyzed during enzyme assay with enzyme solution of pH 5 and pH 6

The activity of the enzyme is maximum in the initial time of the reaction and when reaction proceeds the activity reduces and hence the product concentration of GlcNAc reduces from day 1 to day 6. The maximum GlcNAc is obtained with enzyme solution of volume 5 mL. It is represented in below graph 4.



Graph 4. GlcNAc concentration (g/L) during hydrolysis of CC by using enzyme solution of pH 5



Graph 5. Protein concentration (g/L) during hydrolysis of CC by using enzyme solution of pH 5

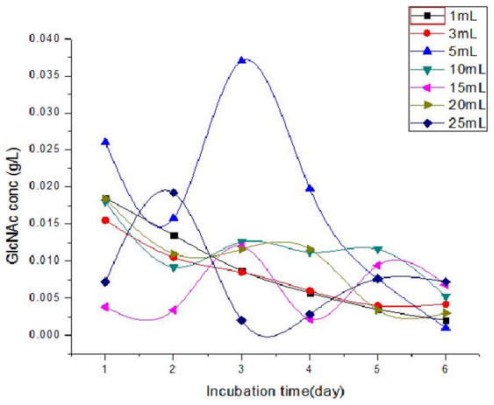
The proteins released during hydrolysis increases with time and maximum proteins are released in the hydrolysis media on the sixth day with enzyme solution of pH 5 with concentration of 5 mL followed by 10 mL and 15mL. It is represented in below graph 5. Similarly the experiment was carried for pH 6, Here also the activity of enzyme decreases with time and maximum result is obtained during hydrolysis of colloidal chitin with enzyme solution of concentration 5 mL. When the results of GlcNAc produced by enzyme solution of pH 6 compared to that of products of enzyme solution of pH 5, the results are maximum and considerably good with enzyme solution of pH 6. The amount of protein released during the hydrolysis of colloidal chitin increases according to the incubation time. The maximum results are observed on the sixth day and with inoculum concentration of 25 mL, the maximum amounts of proteins are released followed by 5 mL inoculum concentration and when compared to those results of proteins released with enzyme solution of pH 5, the results of enzyme solution of pH 6 are comparatively higher, as shown in graph 6 and 7.

Substrate concentration dependency on hydrolysis of colloidal chitin for the product formation

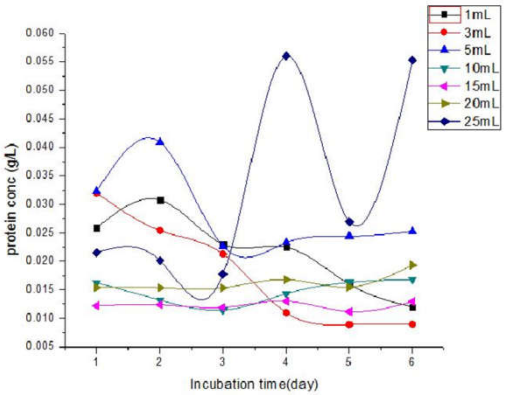
It was conducted for six days and results are analyzed after every 24 h for estimation of product released during the hydrolysis. The graph implies that the variation in substrate concentration affects the formation of the GlcNAc and product formation gradually increases according to the incubation time. The maximum product formation is seen in the hydrolysis media containing 2 g of colloidal chitin. It is represented in the graph8. The results were analyzed for determining the amount of protein released during hydrolysis of colloidal chitin of various concentrations. And the results are shown in Graph 9. The amount of protein is higher with substrate concentration of 3 g followed by the amount of protein released by the hydrolysis media containing 2 g and 1 g of colloidal chitin.

Effect of varying pellet number and agitation intensity on hydrolysis of colloidal chitin

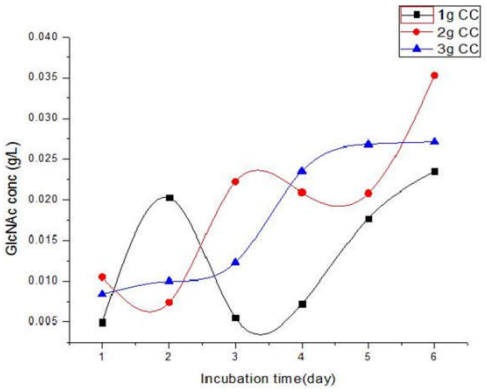
Experiment is carried out for six days by inoculating the media with seed media pellets like 5, 10 and 15 at 130 rpm. The samples are collected every day and analyzed for formation of product GlcNAc.



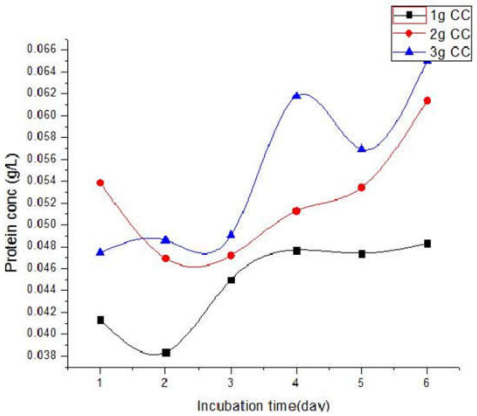
Graph 7. Protein concentration (g/L) during hydrolysis of CC by using enzyme solution of pH 6



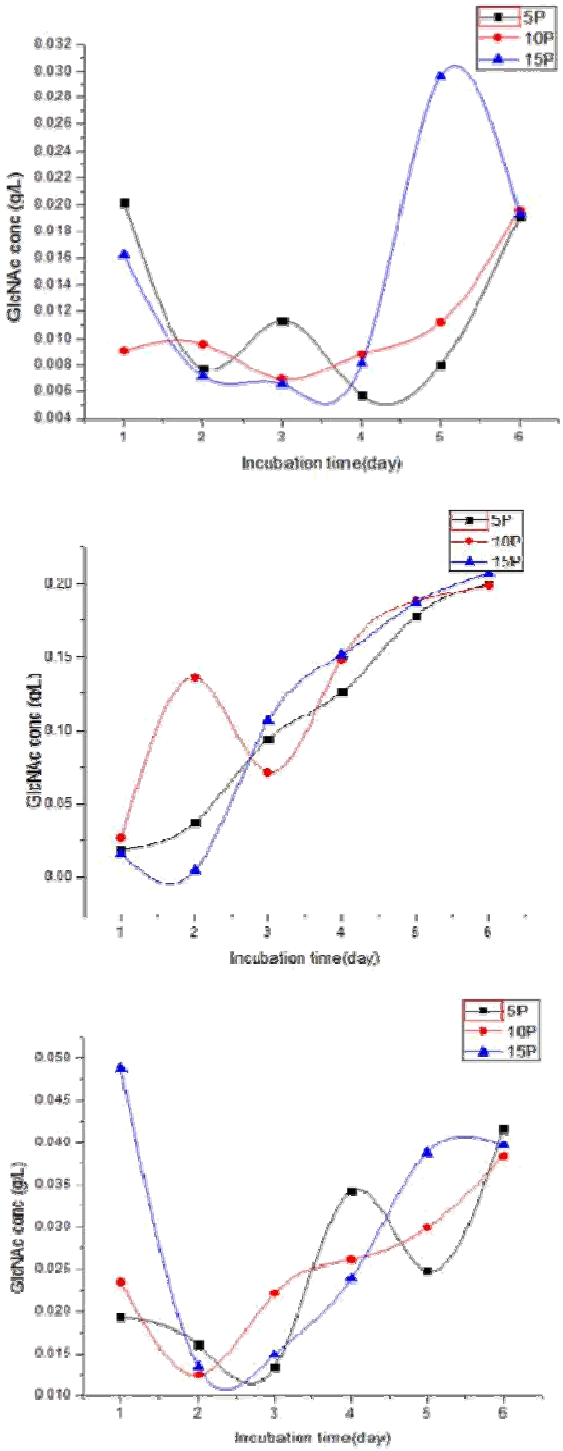
Graph 6. GlcNAc concentration (g/L) during hydrolysis of CC by using enzyme solution of pH 6



Graph 8. GlcNAc (g/L) concentration of hydrolysis media containing chitin in varying concentration

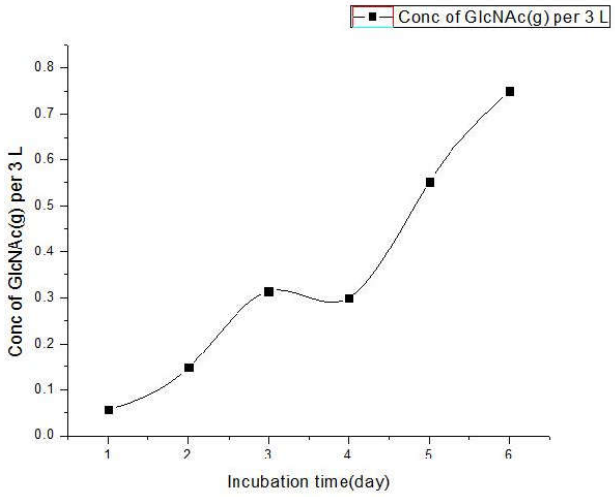


Graph 9. Protein (g/L) concentration of hydrolysis media containing chitin in varying conc

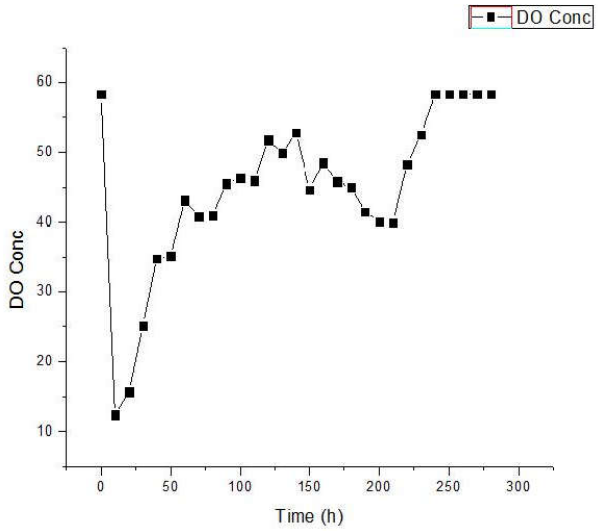


Graph 10. GlcNAc (g/L) concentration of hydrolysis media at a) 130, b) 180, c) 210 rpm

The values plotted on graph imply that the production of GlcNAc depends on number of pellets because the concentration of inoculum varies with different number of pellets. And the results show that the concentration of GlcNAc is considerably maximum in hydrolysis media containing 15 pellets. It is represented in the following Graph10a. The second part is conducted by inoculating the hydrolysis media with same number of pellets like 5, 10 and 15 at 180 rpm. Here also the concentration of GlcNAc increases with number of pellets. It implies that the hydrolysis media inoculated with 15 numbers of pellets produces comparatively more amount of GlcNAc during the hydrolysis because of increased concentration of inoculum. Results are represented in the below Graph 10b.The third part is conducted by inoculating the hydrolysis media with same number of pellets like 5, 10 and 15 at 210 rpm. Here also the concentration of GlcNAc increases with the different number of pellets and it is observed that maximum amount GlcNAc is produced in the reaction mixture containing 5 and 15 pellets followed by the hydrolysis media inoculated with 10 numbers of pellets. Results are represented in the below Graph 10c. When individual experiments are observed, the results on sixth day at same rpm with different pellets are almost close to each other and it is true in all the three rpm levels. Hence it can be concluded that agitation intensity does not affect the number of pellets on formation of product and 180 rpm level is effective agitation intensity to get the good results.



Graph 11. GlcNAc formation by T. harzianum during hydrolysis of colloidal chitin in bioreactor



Graph 12. Concentration of DO against time of aeration

Volumetric oxygen transfer coefficient during hydrolysis colloidal chitin in lab scale bioreactor

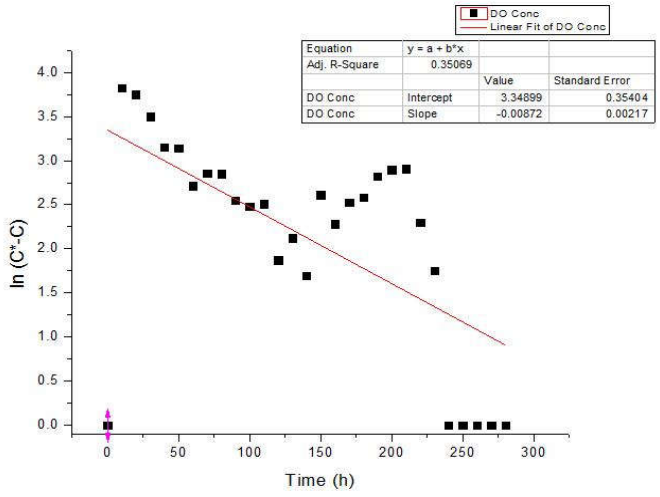
The hydrolysis of colloidal chitin is carried out in lab scale fermenter of capacity 5 L with working volume of 3 L and reaction is carried out at 32 , pH5, 180 rpm with continuous air purging. Samples are collected and analyzed for production of GlcNAc. The formation of GlcNAc during hydrolysis of

colloidal chitin in the lab scale bioreactor increases in accordance with the incubation time which is represented in the graph 11. The maximum concentration of product is obtained on the 6th day of the experiment. Mass transfer coefficient is determined by using of dynamic gassing out method where the nitrogen gas is sparged to reduce the dissolved oxygen level close to zero. The initial DO level should be noted down before sparging of nitrogen. The level of DO reduces close to zero, air is supplied to increase the level of DO and rate of increase is recorded. Even after the DO reaches a constant condition, purging is continued for about 30 h to confirm equilibrium condition. Saturation DO is used to calculate volumetric oxygen coefficient. Variation in DO during air supply against the incubation time is recorded. The level of oxygen concentration increases gradually with time of incubation, and the purging is terminated when the saturation is attained. Time versus DO concentration is shown in Graph 12. Further, the mass transfer coefficient (k_La) is calculated according to the following equation;

$$\ln (C^*-C) = \ln (C^*-C_0) - k_L a.\ t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

C* is saturation concentration of oxygen C is oxygen concentration in the liquid

C_0 is oxygen concentration at t = 0.



Graph 13. Plot of in (C*-C) Vs time

The volumetric mass transfer coefficient is determined by plotting ln (C*-C) against time. Saturation concentration is found to be equal to 58.3. The values are represented in the Graph 13.

The slope of line is -0.008. The negative value represents ln (C*-C) decreases with time. The mathematic equation for straight line is given by,

$$y = mx + c \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

By comparing the above two equation the k_La is equal to slope m, and hence the mass transfer coefficient is 0.008 m/h.

Conclusion

The experiments of hydrolysis are carried out by varying different parameters such as inoculum concentration at different pH, substrate concentration, pellet numbers and speed of the orbital shaker. Hydrolysis experiments are carried out with enzyme solution of pH 5 and pH 6, good results are obtained with enzyme solution of pH 6 and when the results are compared with product obtained during hydrolysis of colloidal chitin inoculated with seed media (pellets), GlcNAc concentration are observed to be maximum during the hydrolysis with enzyme solution. This is due to highest activity of the enzymes at the initial stage of reaction. Experiments are carried out in order to check the substrate concentration dependency by varying concentration of colloidal chitin. 1 g, 2 g and 3 g of substrate is added in different conical flasks containing hydrolysis media, the hydrolysis is carried out for six days and maximum amount of product GlcNAc is obtained during the degradation of chitin containing 2g of colloidal chitin in the media on the sixth day. Enzymatic hydrolysis is carried out using the inoculum containing different number of pellets like 5, 10 and 15. Further effect of agitation is studied by varying speed of orbital shaker. Experiments are carried out at agitation speed of 130 rpm, 180 rpm and 210 rpm with same number of pellets. Variation in the pellet number is not affected by the speed in the orbital shaker and highest GlcNAc is at speed of 180 rpm. Hydrolysis is carried out in a laboratory scale bioreactor at pH 5, 32 temperature and mixing intensity of 180 rpm and at different environment like probes of air sparger, temperature sensor compared to conical flask reactors. The samples are analysed for product concentration. As oxygenation is important parameter, the volumetric oxygen transfer coefficient is calculated by "dynamic gassing out method". The calculated value for volumetric oxygen transfer coefficient is 0.008 m/h.

REFERENCES

Acharya, M. M. and Katyarem S.S. 2004. An Improved Micromethod for Tyrosine Estimation, Z. *Naturforsch*.,vol59, pp897-900.

Cirano J. Ulhoa and John F. Peberdy, 1991. Regulation of Chitinase in *T. harzianum*, *Journal of General Microbiology,* Vol 137, pp 2163-2169.

Felse, P.A., Panda, T. 1999. Studies on applications of chitin and its derivatives, *J. Bioprocess Engineering*, Vol 20, pp505-512.

Jayakumar, R., Prabaharan, M., Kumar, P.T.S. 2011. Biomaterials based on chitin and chitosan in wound dressing applications, *Biotechnol. Adv*.,Vol29, pp323-325.

Kapat, A., Rakshit, S. K., Panda, T. 1996. Parameters optimization of chitin hydrolysis by *Trichodermaharzianum* chitinase under assay conditions, *Bioprocess Engineering Journal,* vol14, pp275-279.

Majeti N.V. Ravi Kumar, 2000. A review of chitin and chitosan applications, Reactive & Functional Polymers, Vol 46, pp1–27.

Mako, G.A., Alimova, F.K. 2006. Growth Kinetics of T.harizanium in green house, *Biotechnol. Adv*., Vol. 3, pp280-283.

Maria Papagianni, 2014. Characterization of fungal morphology using digital image analysis techniques, *Journal of Microbial and Biochemical Technology*, vol. 6(4), 2014, pp189-194.

Michele Michelin *et al.*, Influence of volumetric oxygen transfer coefficient (kLa) on xylanases batch production by *Aspergillusniger*van Tieghem in stirred tank and internal-

loop airlift bioreactors, *Biochemical Engineering Journal*, Vol. 80, 2013, pp19-26.

Miller, G.L. 1959. Use of dinitrosalicylicacid reagent for determination of reducing sugar, *Anal. Chem*., vol31, pp 426-428.

Nickos A. Botsoglou, Dimitrios J. Fletouris, Georgios E. Papageorgiou, Antonios J. Mantis, 1993. Derivative spectrophotometric method for the analysis of tyrosine in unhydrolyzed protein, food, and feedstuff sample, vol41(10), pp1635-1639.

OlaGomaa, M. and A. Heba El Bialy, Pellet Morphology, 2009. Broth Rheology and Statin Production in Submerged Fermentation of P. citrinum, *Global Journal of Biotechnology and Biochemistry*, vol 4 (2), , pp 75-83.

Patil, R.S., Ghormade, V., Deshpande, M.V. 1999. Chitinolytic enzymes: an exploration, Enzyme Microb. Technol., Vol26, pp473–483.

Pradip Kumar Dutta, Joydeep Dutta and V.S. Tripathi, 2004. Chitin and Chitosan: Chemistry, Properties and Applications, Department of Chemistry, *Motilal Nehru National Institute of Technology,* Vol 63, pp 20-31.

Rodde, R.H., Einbu, A., Varum, K.M. 2008. Seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (Pandalus borealis), Carbohydr. *Polym.*, Vol. 71(8), pp388-393.

Rodriguez Porcel, E. M., Casas Lopez, Sanchez Perez, J. A, Fernandez Sevilla, J. M, Chisti, Y. 2005. Effects of pellet morphology on broth rheology in fermentations of *Aspergillusterreus, Biochemical Engineering Journal*, Vol 26, pp139-144.

Subramanya, C., Rao, S.L.N. 1987. An enzymic method for the determination of chitin and chitosan in fungal cell walls, *J.Biosci.,*Vol12, pp125–129.

Syahiddin Dahlan Said, 2007. Spore Production by Biocontrol Agent Trichodermaharzianumin Submerged Fermentation: Effect of Agitation and Aeration, *Journal Rekayasa Kimiadan Lingkungan,* Vol. 6, pp 71-76.

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