# PRODUCTION OF RECOMBINANT CHITINASE FROM *Trichoderma virens* UKM-1 IN *E.coli*

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# Chitin

- An insoluble β (1-4)-linked polymer of N-acetyl glucosamine.
- A major component of the cuticle and the gut lining or peritrophic membrane of insect.
- An abundant renewable natural resource obtained from marine invertebrates, insects, fungi and algae.
- The most abundant polysaccharide in nature and a major constituent of the cell walls of many fungi, insect exoskeletons, and crustacean shells.

# Chitinases

- Enzyme capable of hydrolyzing chitin to its monomer N-acetyl glucosamine.
- Chitinases are found in bacteria, fungi, virus, and higher plants.

# Application of Chitinases

- In biocontrol of plant pathogenic fungi and insects
- Mosquito control
- Biopestisides
- Degradation of fish waste
- In the manufacture of chemical and pharmaceutical intermediates and food product.

# Objective

- The main objective of this study is to enhance the production of recombinant chitinase from *Trichoderma virens* UKM-1 in *E.coli* in the form of soluble/active protein.

# Scope of study

- To observe the optimum post induction time, by varying the time sample taken. Induction times ranging from 0 to 10 h at 2 h intervals.
- To observe the optimum growth temperature for chitinases to be expressed by vary the temperature at 18, 20, 22 and 30$^{o}$C.
- To observe the optimum initial pH of the medium culture to use in order to get the optimum expression of chitinase through varying the pH of the medium between pH 6, pH 7 and pH 8.
- To determine chitinase activity from the expression enzyme

# Methodology

*E.coli* cells were cultivated in 10 ml LB medium with ampicilin (16-18 hours at 37°C , 200 rpm)

10% of culture of *E. coli* was grown in 250 ml flask containing of 50 ml LB medium.

The culture was incubated at 370C with shaking until A600 of the bacterial culture was 0.4-0.5

IPTG was induced to a final concentration of 1.0 mM.

# Cont’

Further incubate at 18, 20, 22, and 30°C.Flasks were removed every 2 h.

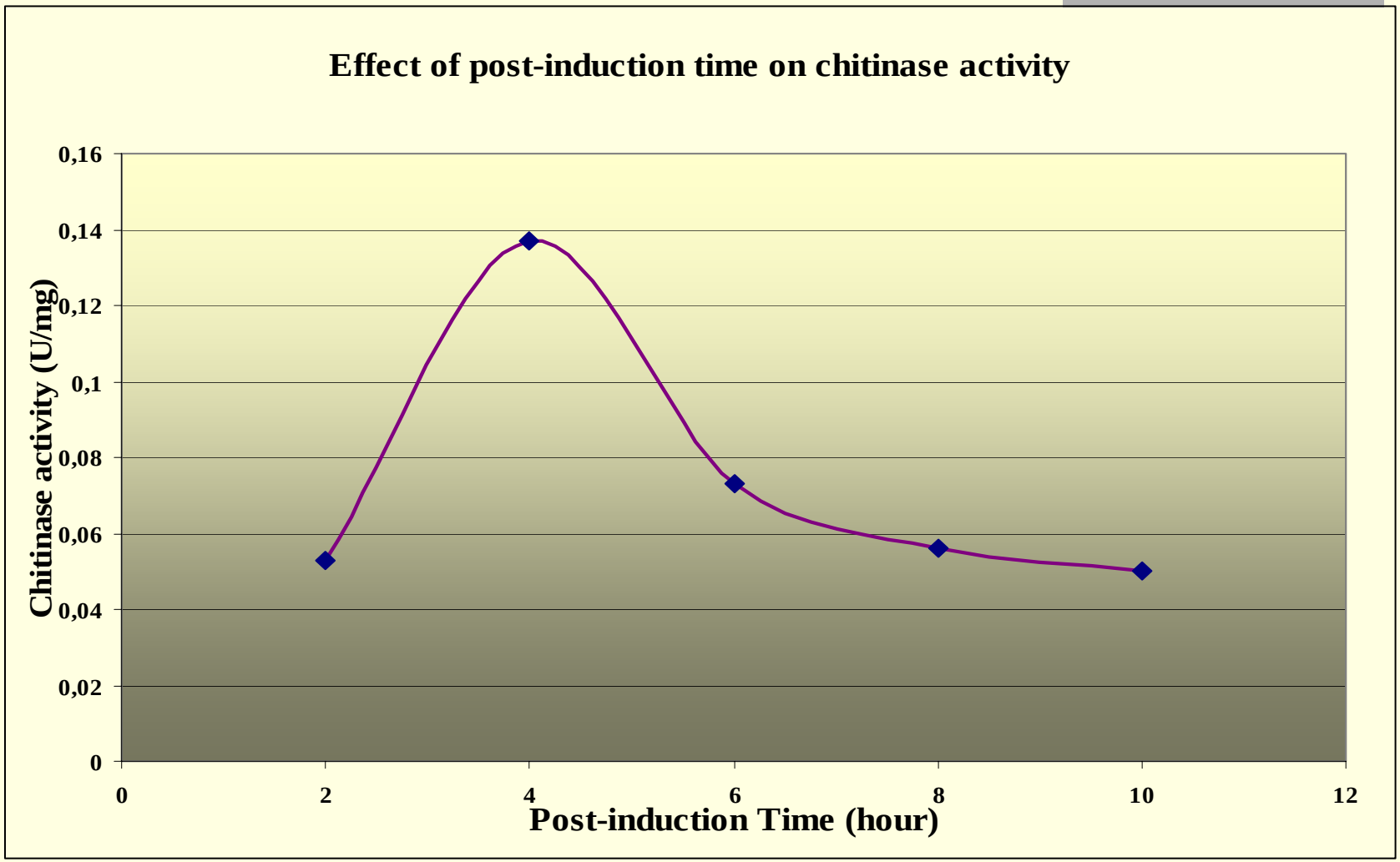
Harvest cells by centrifugation at 8000g, 4°C for 10 min. Pellet was taken

Cell disruption by Lysozyme.

Determination chitinase activity by using DNS method

# Results and Discussion

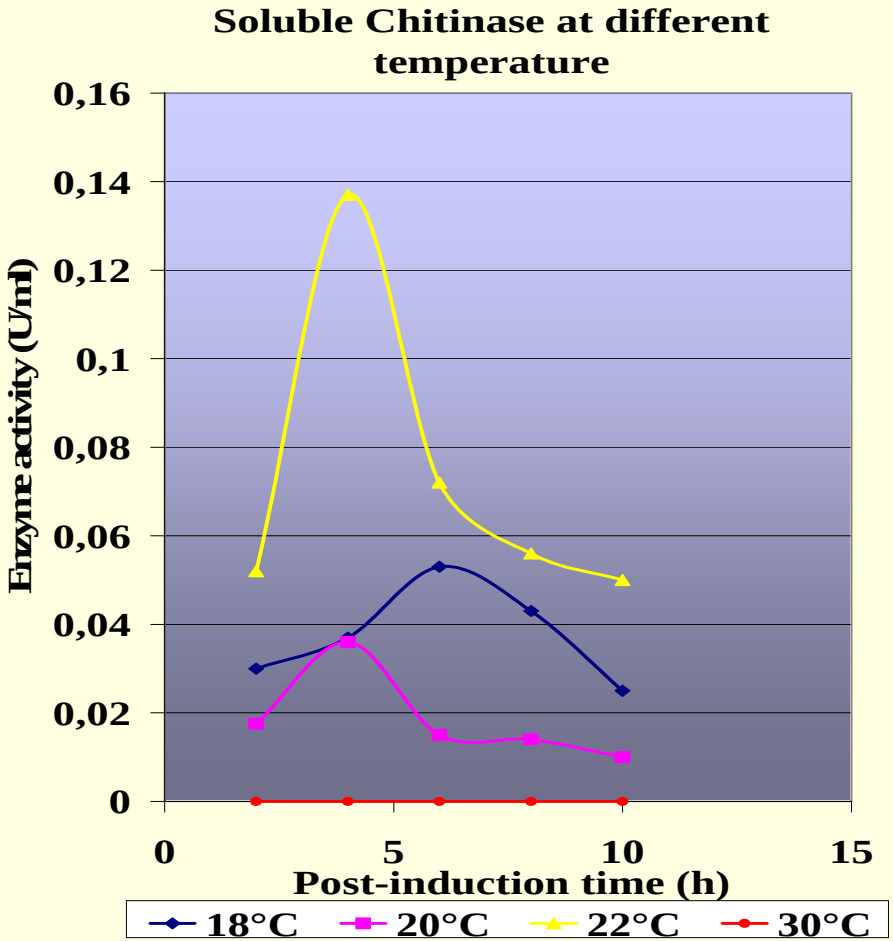
# Optimization of post induction time



# Cont’

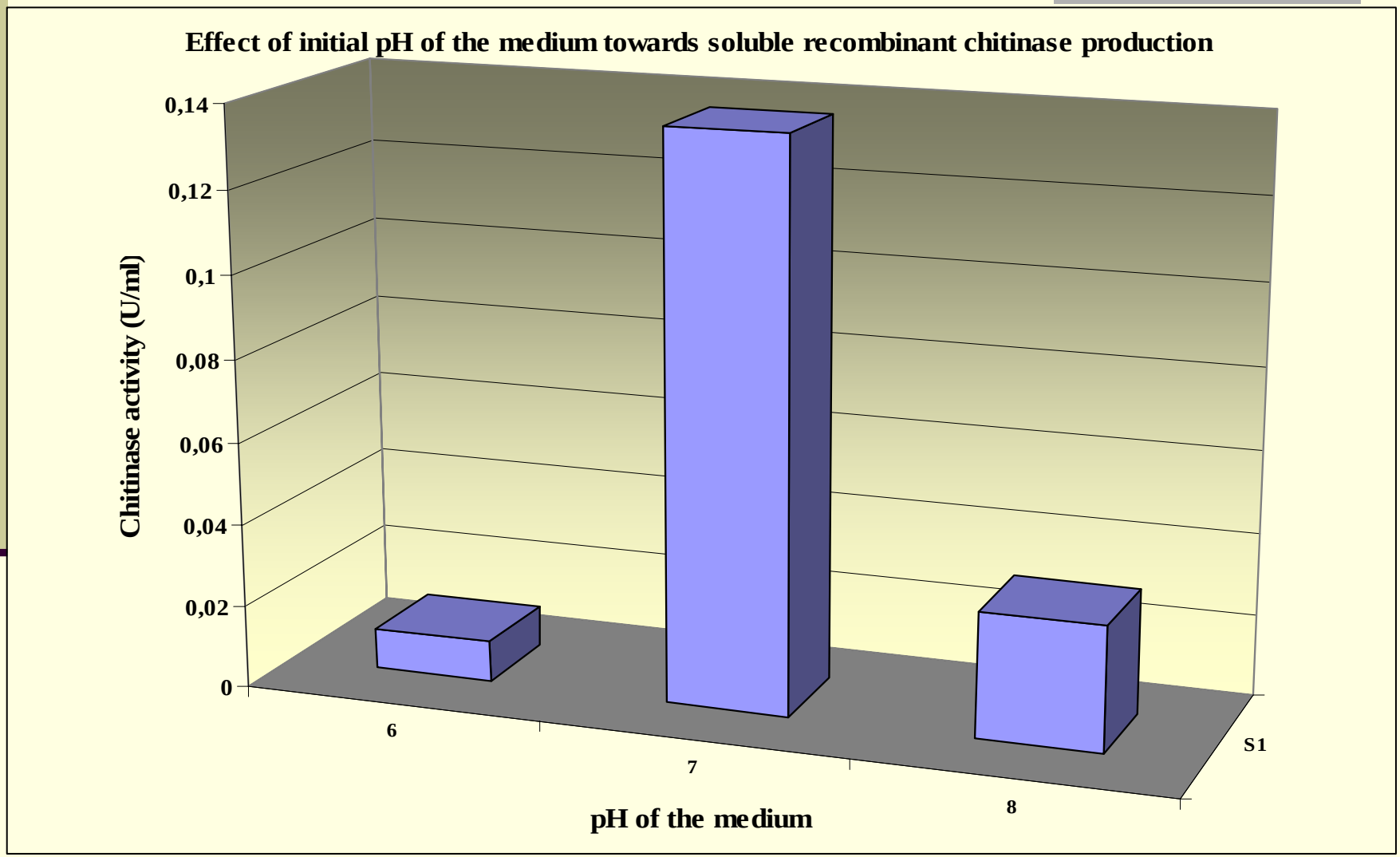
- The enzyme production showed growth relatedness.
- maximum enzyme production was observed after 4 h.
- After 4 h, the production started to decrease. The organism could no longer balance its steady growth with the availability of nutrient resources.
- Similar result was observed on the production of T1 lipase from *Geobacillus* sp in *E.coli (*Raja Noor Zaliha *et al* 2005*)*

# Optimization of incubation temperature




- Strong influence of incubation temperature on active chitinase yield by *E.coli* .
- Higher chitinase activity = 22°C .
- No chitinase activity at 30° C could be due to the formation of inclusion bodies
- Soluble enzyme was perform at lower temperature to minimize inclusion body formation

# Optimization of initial pH of the culture medium



# Cont’

- The enzyme exhibited an optimum activity at pH 7.0
- This result is an agreement with chitinase from *Aeromonas caviae* (Chung-Saint Lin *et al* 1997**)**.
- Most of the fungal chitinolytic enzymes have pH optimum between 4.0 and 7.0. (katatny *et al* 2001).

# Conclusions

- The optimum of enzyme induction period was found to be 4 h culture time.
- Expression at 22°C had lead to an optimum production of soluble chitinase.
- Recombinant chitinase from *Trichoderma virens* UKM-1 expressed in *E.coli* required slightly neutral pH to perform its expression optimally.

# Recommendations

- Using statistical method to optimize production.
- Scale up production using 5 or 10 liter fermenter.

THANK YOU ☺