# Optimization of Temperature and Cellulase pH from Rumen Bacteria Isolation of Beef Cattle

**Mirni Lamid[1], Ni Nyoman Tri Puspaningsih[2], and Widya Paramita L[1]**

[1] Department of Husbandry, Faculty of Veterinary Medicine, Airlangga University; [2]Department of Chemistry, Faculty of Science and Technology, Airlangga University, C Campus, Jl Mulyorejo, Surabaya, Indonesia; e-mail: mirnylamid@yahoo.com

## Abstract

Biotechnology in animal feed using biological treatment such as cellulase is mainly aimed to increase degrade the cellulose; the second most abundant polysaccharide in nature. Information of using cellulase as biocatalyst for increasing animal feed quality as ruminants feed is limited. Cellulose is the major component of cell wall agroindustry waste after hemicellulose. This cellulase comes from *Actinobacillus* sp. that were isolated from rumen fluid of beef cattle. The aim of this research was to determine activity of cellulase and characterization of optimum temperature and pH. In this research identification with carboxyl methylcellulose (CMC) has been done. Methods for cellulase activity was 3,5-dinitrosalicylic acid (DNS). The results showed that *Actinobacillus* which have positive activity of cellulase. Characterization of *Actinobacillus* sp. had optimum temperature 45 °C with activity 1.42 U/ml and optimum pH 6 with activity 0.57 U/ml.

**Key words:** cellulase, carboxyl methyl cellulose, temperature, beef cattle

## Introduction

The potential of agro industry waste product is great, but the problem occurred was the low value of nutrition signed the high content of crude fiber (cellulose, hemicelluloses, and lignin) and the low value of crude protein. Cellulose and hemicelluloses provided used a little part only and the most become waste product. It is caused cell wall of agro industry waste product realized lignifications, make a complex bound with lignin. Cellulose as an organic material compound abundant in this nature has a sharp filament like; it is content of cell wall of plant. Cellulose is the highest component of the plant. Cellulose is the glucose polymer connected with glycoside no branch. Most of animal can not digest cellulose because they don't have cellulose enzyme in their digestive tract. Ruminant can use cellulose as their nutrient source because in their rumen contain cellulolitic bacteria produced cellulose enzyme to hydrolyze cellulose (Campbell, 1994).

Cellulose molecule is the polysaccharide with bound ß-1-4 glycoside that is difficult to digested by rumen bacteria therefore digestibility of cellulose was very low (Mc. Donald *et al.*, 1995). Rumen microbes are main source of cellulose enzyme producer but digestibility of agro industry waste still low so need a process before. Utilizing of cellulose enzyme group will increase nutrition value of agro industry waste product. Cellulose enzyme contains 3 enzyme components, that is: component C1 (β-1, 4-glucan cellobiohydrolase or exo-β-1, 4-glucanase), component Cc (endo-β-1, 4-glucanase) and component cellobyose (β-glucocidase). The centre point of cellulose degradation provide in the break of bound 1.4 β-glycoside. The breaking of bound 1.4 β-glycoside caused cellulose hydrolyzed become simpler component that is oligosaccharide (mainly cellobyose). Then it will hydrolyze polysaccharide (mainly glucose). The breaking of 1.4 β-glycoside bound done by cellolase enzyme group (Bondy, 1987).

Improvement of hydrolyze enzymatically was a new prospect to handle ligno cellulose become the feed for ruminant. Biodegradation of cellulose enzymatically accomplished by cellulose enzyme produced by much kind of microbes, such as: fungi, bacteria or *actinomycetes, alga, oomycetes*, ruminant (Campbell, 1995).

Cellulose enzyme has very big industry application that is textile industry, pharmacy, paper and pulp, food and animal feed, and chemistry industry. Application of cellulose enzyme becomes one of component in chicken rations (Sunna and

Antranikian, 1997). Cellulose enzyme gather with xilanase can break glucose reduce the fermentation result of structural polysaccharide of the plant cell wall at the grass cellulase process, thus utilizing of this enzyme can increase consumption and digestibility of ruminant (Spoelstra *et al.,* 1990). The giving of feed additive enzyme manly cellulase and xilanase can increase feed efficiency for beef cattle and dairy cattle (Beauchemin *et al.,* 2003).

The protein structure of enzyme can influence catalytic activities. Catalytic activities of enzyme will be loss if protein denaturizing occurred. The occurring cause of protein denaturizing is extreme pH, high temperature, and influencing of other component such as detergent, ionic, organic solution, concentrate urea, big anion of strong acid and chaotropic ion (I-,SCN-)(Ottoway and Apps, 1984). Considered from the biochemistry structural, there are some difference between enzyme termophilic and mesophilic. Beside from disulfide content, hydrophobic interaction level at the molecule has enzyme stability. The optimum temperature of termophilic enzyme activities is about 50$^{o}$-80$^{o}$ C, and for termophilic enzyme is at the room temperature, about 25$^{o}$-50$^{o}$ C. if the mesophilic enzyme placed at higher temperature will realize denaturizing.

This research used bacteria isolation from beef cattle rumen, *Actinobacillus* sp. The research was to investigate cellulase activities then accomplished characterization to determine optimal temperature and pH of cellulose enzyme from the bacteria isolation.

## Materials and Methods

### Sample

The sample used was bacteria isolation, *Actinobacillus* sp. produced from beef cattle rumen.

### Production of fluid media

Fluid media contains of 0.2 g trypton; 0.1 yeast extract; 0.2 g NaCl. All materials dissolved at the Erlenmeyer tube 100 ml with 20 ml aquadest, and then closed by cotton and aluminum foil. After that it sterilized by autoclave for 45 minutes at 121°C, 1 atm.

### Production of solid media

Fluid media contains of 0.2 g trypton; 0.1 yeast extract; 0.2 g NaCl; o, 3 g bacto agar. All materials dissolved at the Erlenmeyer tube 100 ml with 20 ml aquadest, and then closed by cotton and aluminum foil. After that it sterilized by autoclave for 45 minutes at 121$^{o}$C, 1 tam. Then waited until lukewarm and poured into Petri disk then let it until cold and hard or solid.

### Halo test

Bacteria isolation, *Actinobacillus* sp. planted in the solid media contained substrate Carboxyl Methyl Cellulose (CMC) by streak ose to the bacteria colony then streak again to the surface of solid media. Incubated at 37°C for 16 hours. Observation done to find Halo (transparent area) around the colony then choose the isolation had the biggest Halo and also shown the highest cellulase activities.

Then Halo gotten was examined by Congo-red 0.1% poured in to the isolation. Positive result signed with Halo area transparent still and the color became red (Puspaningsih, 2004).

### Inoculums production

Isolation renewed was moved to the growth media 20 ml in the Erlenmeyer tube 100 ml, and then incubated with shaking 150 rpm at 39°C during optimal cultivation time received from growth curve. Cultivation result was cell suspension or inoculums

### Enzyme production

Enzyme production accomplished by inoculated 1% inoculums in to 50 ml fluid media contained carboxyl methyl cellulose (CMC) in the Erlenmeyer tube 250 ml and incubated at 39°C for 16 hours with shaking 170 rpm. Incubation result taken the supernatant for tested the cellulase enzyme activities.

### The cellulase activities tested by method 3,5-dinitro-salicylic (DNS) acid

A number 100µl enzyme and xilan substrate entered to the each Eppendrof tube then incubated at 39$^{o}$C in the water heater for an hour. For control, the formula was same with sample without incubation. Then sample and control added 600µl DNS, after that heated in the boiled water for 15 minutes, then incubated in the ice bath for 20 minutes. Absorbance read in the wave length 550nm. 1 unit enzyme activities defined as a total enzyme produced 1µmol xylosa per minute for every ml enzyme, and the specific activities was enzyme unit divided protein content (Miller, 19960).

### Characterization of Cellulose Enzyme

Determination of optimum temperature for cellulase done by determined activities of cellulase at many kind temperatures variant, that was at temperature: 35, 40, 45, 50, 55 and 60°C.

Determination of optimum temperature of cellulase determined by activity assay to cellulase at pH 5-9 used buffer phosphate citrate at optimum temperature of cellulase.

## Results and Discussion

The research result of enzymes cellulase activities from bacteria *Actinobacillus* sp. planted in the medium contained MCM at the many kind of temperature presented at Table 1 and Fig. 1. The influence of temperature on the cellulase activities at this result investigated at 35-60°C. Optimization temperature result of cellulase received at 45°C with activities 1.42 U/ml, but at 50°C and 55°C cellulase still have good activities that reached 92.96% (1.32 U/ml) and 78.17% (1.11 U/ml). At the temperature above 55°C decreasing of cellulase activities occurred, because atoms in the enzyme molecule had big enough energy to move, it caused by the presence form changing of enzyme structure because increasing thermal tremble of atoms component, therefore protein as enzyme material denaturized.

The research of optimum temperature was lower then the research result reported by Lowe *et al.*, (1997) that rumen fungi *Neocallimastix frontalis* plant at the wheat straw substrate produced cellulase had optimum activities at 50°C.

Cellulase activities realized increasing from pH 5 reached the highest activities about 0.57 U/ml at pH 6, at pH 7 cellulase activities decreased reached 73.68% (0.42 U/ml) from activities at the optimum pH. It's probably caused by conformation changing between enzyme and substrate because pH decreasing (Fig. 2).

Generally, in the cattle rumen the optimum pH was 6-7. According to the research result, gotten optimum pH from cellulose enzyme was suitable with condition of rumen pH. It was one of cellulase characteristic that was total ions at that pH made confirmation of cellulase formed a pair with the substrate. It caused also by enzyme received from rumen bacteria, thus pH gotten was suitable with bacteria's habitat. Optimum condition at pH 6-7 was suitable to growth cellulitic and hemicellulitic bacteria in the rumen to degraded fibrous feed produced VFA. This result synchronizes with the opinion of Owens and Goetsch (1988) that pH condition very influenced by microbes environmental in the feed degradation. That cellulolitic and hemicellulolitic bacteria need pH about 6.2-7.0 to develop fast (Orskov and Ryle, 1990).

**Table 1.** The Data of Optimum Temperature of Cellulase of Bacteria *Actinobacillus* sp.

| Temperature (°C) | Cellulase activities (U/ml) |
|---|---|
| 35 | 0.86 |
| 40 | 0.71 |
| 45 | 1.42 |
| 50 | 1.32 |
| 55 | 1.11 |
| 60 | 0.96 |

**Table 2.** The Data of Cellulase Optimum pH of Bacteria *Actinobacillus* sp.

| pH | Cellulase Activities (U/ml) |
|---|---|
| 5 | 0.53 |
| 6 | 0.57 |
| 7 | 0.42 |
| 8 | 0.16 |
| 9 | 0.15 |

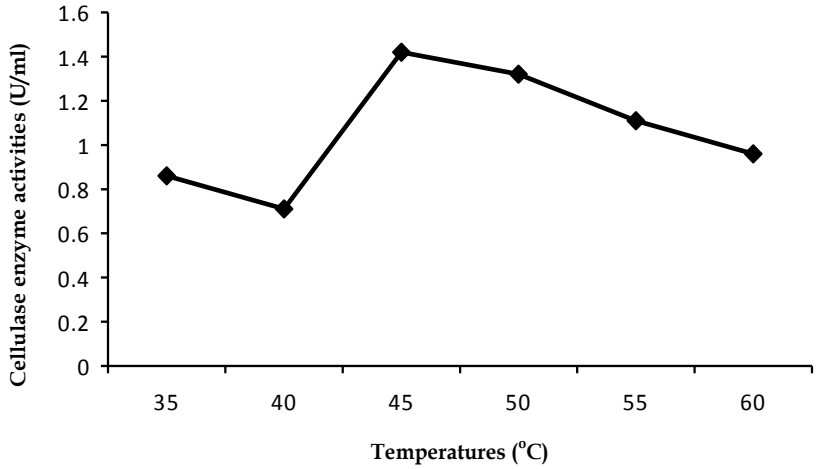


**Figure 1.** The pattern of relationship between temperature and the cellulase enzyme activities.

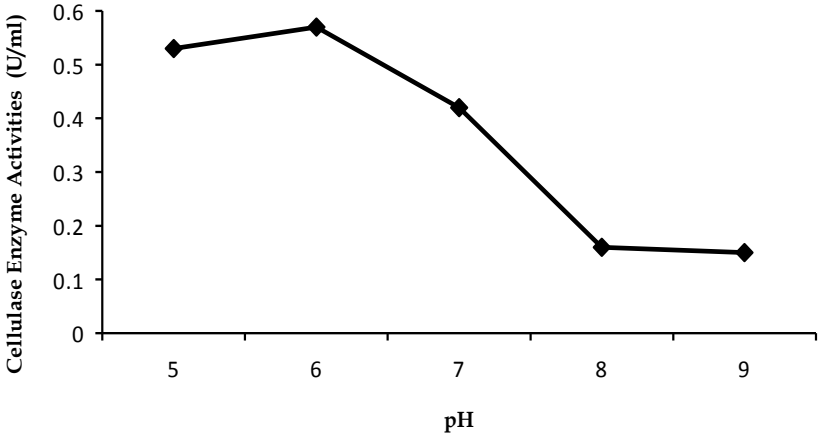


**Figure 2.** The pattern of relationship between pH and the cellulase enzyme activities.

## Conclusion

The characterization of cellulase enzyme has optimum temperature 45°C about 1.42 U/ml and optimum pH about 0.56 U/ml

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