Niken Prawesti Listyaningrum. 146100100111006. Isolation and Characteristic of Enzime Cellulase from *Bacillus* sp. 13843 B15 dan *Bacillus licheniformis* C55. Thesis. Supervisors: Dr. Agustin KrisnaWardani, S.TP, M.Si and Dr. Ir. AjiSutrisno, M.Sc

SUMMARY

Solid waste of Carrageenan is one of waste that has not been utilized and it became the problem of environmental pollution since the waste contains cellulose which is unsoluble in water. Solid waste of carrageenan industry has been utilized biometrics filler, composite amplifier and fertilizer for plant. Isolation of cellulolytic microbes of carrageenan waste is expected to be better in decomposing cellulase in carrageenan waste. This research aims to find out isolates that produce cellulose which is isolated from carrageenan waste and characteristic of isolated cellulase enzyme.

The steps of this research include microbe isolation from carrageenan waste, identification of selected isolate, characteristic of enzyme and cellulase application from selected isolate and the uttilazion of carrageenan waste as component to produce cellulase. Selected isolate identification included identification morphologically, biochemical and molecular that used 16S rRna. Enzyme characterization consisted determining molecule mass using SDS-PAGE and zymogram, effect of temperature and PH toward enzyme activity and metal ion effect toward enzyme activity. Obtained data was then analyzed in quantitative descriptive.

Result of this research was obtained 2 bacteria, B15 and C55. This isolate growth in media of Ph 8 in temperature of 50°C, was positive gram formed stem. Based on the result of sequencing encoding gene of 16S rRNA of isolate B15 is 1393 pairs bases that were obtained 99% similarity with *Bacillus* sp. 13843 bacteria and isolate C55 of 1397 pairs that were obtained 100% similarity to *Bacillus licheniformis* bacteria. Cellulase enzyme characteristic of rough isolate *Bacillus* sp. 13843 B15 had optimum activity in Ph 8 of 0,72 U/mL and incubation temperature 50°C of 0,62 U/ml. Enzyme was stable toward temperature of 50°C incubation in ph 8 for 30 minutes. There were 3 band cellulase with consideration of molecule weight of 50, 40, and 30 kDa. The specific substrate test of CMC and filter paper resulted enzyme activity 0,35%. The existence of metal ion which could increase cellulase enzyme was Mg^{2+}. Whereas cellulase enzyme characteristic of rough isolate *Bacillus licheniformis* C55 had optimum activity in ph 8 of 0,70 U/ml and incubation temperature 50°C amounted 0,35 U/ml. Enzyme was stable toward incubation temperature of 50°C in ph 8 for 30 minutes. There was 1 band of cellulase by considering molecule weight of 18 kDa. The specific substrate test of CMC resulted enzyme activity of 0,25 U/ml higher than enzyme activity of filter paper that amounted 0,13 U/ml.

The addition of cellulase enzyme of selected isolate had potential in hydrolyze carrageenan waste to result reducer glucose level in carrageenan waste with the treat NaOH 6% of 1,18 mg/ml (isolate *Bacillus* sp. 13843 B15) and 0,76 mg/ml (isolate *Bacillus licheniformis* C55). Utilization of carrageenan waste as component to produce cellulase resulted the highest enzyme activity of 0,43% (isolate *Bacillus* sp. 13843 B15 and *Bacillus licheniformis* C55) of carrageenan waste by treating NaOH 4%.

Key Words: Bacillus, Cellulase enzime, Carrageenan, Thermophilic.