

Thesis Title	<i>Rhodococcus fascians</i> Cell Immobilization in Column Reactor for Limonin Debittering in Orange Juice
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ABSTRACT

The study of the method of limonin degradation in orange juice by immobilized *Rhodococcus fascians* NRRL-B-15096 cell in a packed-bed column reactor. *R. fascians* cell was able to be induced to metabolise limonin by treatment with limonin medium for 2 hours before immobilization. The results of comparison in activity and stability of gel bead among agar, agarose, alginate, acrylamide and kappa-carageenan, found that kappa-carageenan had the highest achievement. Therefore, kappa-carageenan was used as a carrier for cell immobilization. The optimal content of cells and kappa-carageenan was determined to be in the ratio of 1 : 3 by weight. The appropriate diameter of immobilized cell bead and concentration of potassium chloride used for immobilization were 4 mm and 0.5 M, respectively. The values of K_m of the free cells was found to be 14.726 mM while that of immobilized cells was 3.404 mM. And the values of V_{max} of the free cells was 66.006 mM/min while that of immobilized cells was 13.149 mM/min. The half life of immobilized cells stored at 4 °C was longer than 80 days.

The optimal conditions for the reduction of limonin content in packed-bed column reactor were in the range of 20-35 °C , pH 4-7 , and the flow rate of 25 ml/h by using the volume of 48 ml of orange juice per 60 ml of immobilized cell beads. Under the conditions , the limonin content was reduced from 14.51 ppm to 9.76 ppm , or 32.74 % by weight . This was caused by the loss of 10.37 % fructose , 2.46 % glucose , 4.88 % sucrose , 14.81 % citric acid , and 13.81 % ascorbic acid . In addition , the operation of immobilized cells could be continued twice , the relative activity of immobilized cells of the second operating time was over than a half of the first operating time .