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# Optimization of Substrate Conversion in Hollow Fiber Reactor

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## ABSTRACT

The crude jackbean preparation was used as urease to determine the $Km_{(app)}$, $V_{(app)}$, and substrate conversion (%) at different flow rates in a hollow fiber device. It was found that, as the flow rate increases, the $V_{(app)}$ increases, whereas the $Km_{(app)}$ initially decreases and again increases. The maximum substrate conversion (96.25%) was obtained at the lowest $Km_{(app)}$. No significant loss was observed in substrate conversion after 2 mo at room temperature.

**Index Entries:** Hollow fiber reactor; urease; substrate conversion; apparent enzyme stability.

## INTRODUCTION

Hollow fiber immobilization is a potentially valuable method for the study of a soluble enzyme in comparison to other methods. Hollow fiber reactors (HFR) have being used for biocatalytic conversion of various substrates using enzymes or whole cells (*1*). The major advantage of this system is a large ratio of surface area to volume, which enhances the enzyme-substrate contact and substrate conversion. This technique was first suggested by Rony in a theoretical paper (*2*). Since then, several studies have been carried out with different enzymes (*3*). Recently, lipase was extensively studied (*4,5*). However, the problem of substrate conversion and enzyme stability remained of major concern. A simple and reliable

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approach is necessary to overcome these problems for successful commercialization of this technique. The present article describes a very simple and reliable experimental method for the optimization of substrate conversion. Since the flow rate and substrate concentration can be easily controlled in the reactor, these two parameters were selected to optimize the substrate conversion. The crude enzyme preparation was used to maintain its own microenvironment inside the reactor to enhance its stability.

## MATERIALS AND METHODS

Artificial kidney of Cordis Dow Corp., Miami, FL was used as HFR. Jackbeans and other chemicals (LR-grade) were purchased locally.

### Enzyme Preparation

Two hundred grams of Jackbeans were soaked in 0.025*M* Tris-acetic acid buffer, pH 6.5, containing 0.01% mercaptoethanol (washing buffer) at room temperature (28±2°C) overnight. After homogenization, the mixture was kept in the refrigerator (4°C) overnight. The sediment was removed by filtration and centrifugation at 12,000*g* for 20 min. The collected supernatant was used as urease. The enzyme activity (*6*) was calculated as μmol of ammonia produced/min/mg of protein. The total protein was estimated with Biuret reagent after TCA precipitation.

### HFR Preparation

The reactor was washed with washing buffer, and then the enzyme solution was pumped into the shell side. Figure 1 shows the reactor design. One port of the shell side was sealed, and a hydrophobic vent membrane (air filter, 0.5 μm) was attached to another. The washing buffer was passed through the inlet of the lumen side at the flow rate of 100 mL/h until the effluent coming through the outlet of the lumen side became colorless. Then each concentration of substrate was pumped through the inlet of the lumen side at different flow rates. After passing through three reactor volumes, five samples were collected from the outlet of the lumen side at intervals of 1 h. Then the estimation of ammonia was carried out (*6*), and the average value was taken for calculations. After collection of the samples, the reactor was kept for washing overnight at a flow rate of 40 mL/h with washing buffer. The substrate conversion was calculated as the μmol of ammonia produced min/μmol of substrate by the reactor.

## RESULTS AND DISCUSSION

All the experiments were conducted at room temperature. Catalytic activity was absent in the washings. The $K_m$ of soluble enzyme was found

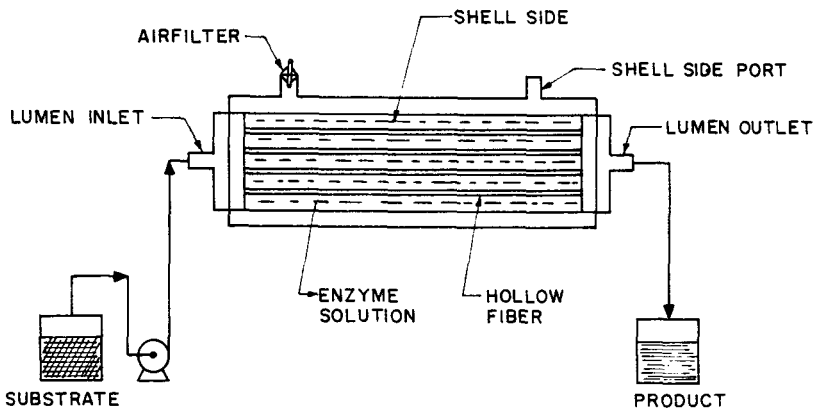


Fig. 1. Schematic of the HFR.

to be 58.4 m*M*. The $Km_{(app)}$ and $V_{(app)}$ were calculated from the Lineweaver-Burk plot. Figure 2 shows the effect of flow rate on $Km_{(app)}$ and $V_{(app)}$ of the reactor. It shows that the $Km_{(app)}$ decreases as the flow rate increases from 40 to 100 mL/h, and it again increases as the flow rate further increases from 100 to 220 mL/h, whereas the $V_{(app)}$ increases as the flow rate increases from 40 to 220 mL/h. This might be because of the effect of some physical factors acting inside the reactor.

Generally, the effect of diffusional resistance decreases as the flow rate increases. The decrease in $Km_{(app)}$ indicated that the effect of diffusional resistance became negligible at flow rate of 100 mL/h, although it might be more at a 40 mL/h flow rate. At a 100 mL/h flow rate, the $Km_{(app)}$ was significantly less (52.5 m*M*) than the $Km_{(app)}$ at other flow rates. This supports the above statement. It was reported that the $Km_{(app)}$ decreased as the flow rate increased (7). The further increase in $Km_{(app)}$ from a 100 mL/h flow rate might be owing to the effect of residence time. The increase in $Km_{(app)}$ only after a 100 mL/h flow rate indicated that the effect of diffusional resistance and residence time became negligible at that flow rate.

In conclusion, all the effects of physical factors acting inside the reactor became negligible as the system approached the 100 mL/h flow rate. This might be the ideal flow rate for maximum substrate conversion. This was further confirmed by the following results. Figure 3 shows the substrate conversion (%) at different substrate concentrations at various flow rates. The maximum substrate conversion (96.25%) was obtained at a 60 m*M* substrate concentration and a 100 mL/h flow rate. At the same flow rate and substrate concentration, the $Km_{(app)}$ was somewhat less (52.5 m*M*) than the $K_m$ of soluble enzyme (58.4 m*M*). This supports the conclusion that the maximum substrate conversion can be obtained by selecting a substrate concentration and flow rate at which the reactor has the lowest $Km_{(app)}$.

The enzyme stability was checked by estimating substrate conversion (%). The 150 m*M* substrate solution was pumped into the reactor at a 100

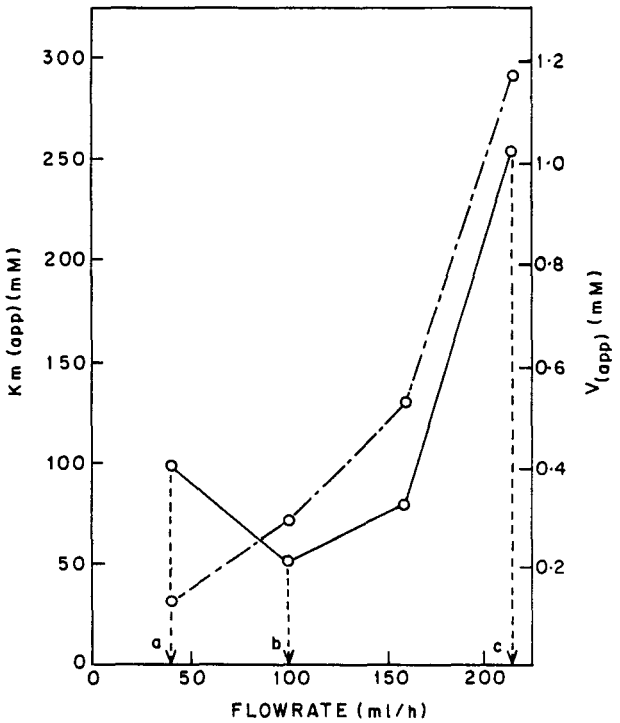


Fig. 2. Effect of flow rate on $Km_{(app)}$ and $V_{(app)}$ of HFR. $Km_{(app)}$ ○—○; $V_{(app)}$ ○———○.

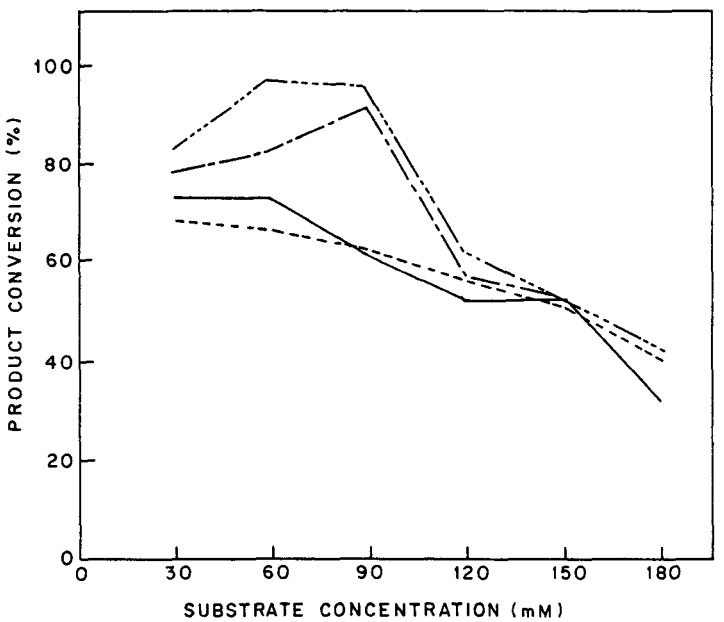


Fig. 3. Effect of flow rate on product conversion at different substrate concentrations. Flow rates: 40 mL/h ——; 100 mL/h ------; 160 mL/h ——; 220 mL/h ---------.

mL/h flow rate. No significant change was observed in substrate conversion at the beginning of the experiment (53.4%) and after 2 mo at the end of the experiment (53%). The reactor was in continuous use for 2 mo. This clearly indicated that the HFR has protected the catalytic power of enzyme. However, it has been reported that the half-life of urease in an HFR was 12 d (*7*) and a crude lipase lost 75% activity in 145 h (*4*). It has been also reported that the half-life of lipase was increased from 40 d to 160 d by changing the polymer material of the hollow fiber. However, the conversion was 70% with 75–85% loss in initial activity (*5*).

The enzyme solution was removed from the shell side of the reactor after 2 mo. In comparison with the original enzyme solution, the removed enzyme solution showed 60% loss in catalytic activity and 91% loss in protein. This might be because of the adsorption of protein inside the reactor. This clearly indicated that the catalytic activity was protected inside the reactor. The use of crude enzyme preparation and presence of mercaptoethanol in the buffer system might have played an important role in the enhancement of the apparent enzyme stability. The chance of microbial contamination in the reactor was ruled out by microscopic examination (gram staining) of removed enzyme solution. After removing the enzyme solution from the reactor, it was washed with 0.85% NaCl, distilled water, and washing buffer, and then the fresh enzyme solution was pumped into the reactor. Almost similar results were obtained. The applicability of the present approach to other system is under study.

## REFERENCES

1. Roy, V. T. B., Blanch, H. W., and Wilke, C. R. (1983), *Trends Biotechnol.* **1,** 135–139.
2. Rony, P. R. (1971), *Biotechnol. Bioeng.* **13,** 431–447.
3. Kitano, H. and Ise, N. (1984), *Trends Biotechnol.* **2(1),** 5–7.
4. Malcata, F. X., Hill, C. G., Jr., and Clyde, H. A. (1992), *Biotechnol. Bioeng.* **39(10),** 1002–1012.
5. Cuperus, F. P., Bouwer, S. T., Krosse, A. M., and Derksen, J. T. P. (1993), *Stud. Org. Chem.* **47,** 269–274.
6. Bernt, E. and Bergmeyer, H. U. (1963), in *Methods of Enzymatic Analysis,* Bergmeyer, H. U., ed., Academic, New York, pp. 401–406.
7. Kitano, H., Yoshijma, S., and Ise, N. (1980), *Biotechnol. Bioeng.* **22,** 2643–2653.