SHORT REPORTS

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CELLULOSE SACCHARIFICATION BY CELLULASES OF *S. CELLULOPHILUM* IN A MEMBRANE REACTOR

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ABSTRACT

The saccharification of cellulose was investigated in a membrane reactor using cellulases of S. cellulophilum. *The amount of total sugar and glucose produced was used to evaluate the effect of permeation rate on the hydrolysis. It was found that changing the permeation rate within the range of 15.1 to 26.0 ml/hr has little effect on cellulose hydrolysis in a 50 ml reactor during the 48-hour experiment. Eighty to ninety percent of the sugar produced was glucose. Enzyme inactivation was observed during a consecutive six-day experiment.*

INTRODUCTION

A group of cellulase complex is required for the effective conversion of agricultural residues to glucose. The soluble products of hydrolysis, i.e. cellobiose and glucose, have been reported to be inhibitors of the cellulase complex.[1] The membrane reactor has been used for cellulose hydrolysis to reduce end-product inhibition.[2-5] The selection of ultrafiltration membranes with specific molecular weight cut-off allows the products or inhibitors to be removed from the system. Moreover, cellulases can be retained in the reactor and reused. Chua[6] has shown that the use of a membrane reactor system in cellulose hydrolysis increases the yield of total sugar 5 - 6 times compared to batch hydrolysis.

Since the rate of permeation through the membrane, which is dependent upon the nature of the solutions and the membrane, can influence the rate of hydrolysis, this communication reports preliminary results on the effect of permeation rate on cellulose hydrolysis in a membrane reactor system. The problem of long-term hydrolysis was also investigated.

The substrate chosen for the study was KC-floc cellulose powder (Sanyo - Kokusaku Pulp Co.). The source of enzyme was crude cellulase of *Sporotrichum cellulophilum*. One gram of cellulose powder was dissolved in 50 ml of 50 mM sodium acetate buffer (pH 5.5) containing 10μg/ml of tetracyclin and 200 μg/ml of streptomycin, then centrifuged at 15,000 rpm for 10 min. The supernatant was used as the enzyme solution.

A flow diagram of the membrane reactor system is shown in Fig. 1. The reactor was UHP-43 (Toyo Roshi Co.) equipped with a membrane (UP-20 Toyo Roshi Co.) of molecular weight cut-off 20,000 and 50.3 cm^2 surface area. Initially, 50 ml of enzyme solution and 5g of KC-floc were directly added to the reactor. Two and a half grams of KC-floc was then added at the end of the first 8 hours and thereafter at the end of every 24 hours of reaction. The buffer solution was pumped from the reservoir into the reactor to keep the reaction mixture constant at 50-55 ml. The permeation rate was varied by adjusting the pressure of the reservoir (0.1 - 0.4 kg/cm^2). The permeate from the reactor was collected at one-hour intervals using a fraction collector. The system was installed in a temperature-controlled room at 30° C.

The product of hydrolysis was analysed for total sugar and glucose using the phenol-sulfuric acid method[7] and glucose oxidase assay (Diacolor-GC, Toyobo Co.), respectively.

Fig. 2. shows the hydrolysis pattern of the 48 - hour experiment done at a flow rate of 20.5 ml/hr. The amount of total sugar and glucose produced increased with time until substrate concentration was low. The addition of KC-floc at 8, 24 and 48 hours resulted in an increase in product yield in the first 4 hours after each addition. During the first 24 hours, the glucose concentration was approximately 65 - 75% of the total sugar. After the first 24 hours this percentage increased to eighty to ninety percent. The high substrate concentration during the first 24 hours might have stimulated cellulase activity and caused the accumulation of cellooligomers as the intermediate product. Then, when the KC-floc concentration was reduced, cellulase activity may have been retarded and cellooligomers were further broken down by β-glucosidase to glucose.

When the rate of permeation was varied from 15.1 to 26.0 ml/hr, the same pattern of hydrolysis was observed, with a slight increase in total sugar when the permeation rate increased. It was observed that the high pressure required to maintain a permeation rate of more than 25 ml/hr caused the cellulose powder to leak into the permeate after the interval addition of substrate. The optimum permeation rate for effective hydrolysis of cellulose powder in the membrane reactor is being investigated.

The hydrolysis experiment was extended to 6 days with new addition of substrate at the end of every 24 hours. The hydrolysis rate was calculated at 8-hour intervals and plotted against time in Figure 3. The changing slopes shown in the figure may indicate enzyme inactivation. If this is the case, the addition of fresh enzyme should restore the hydrolytic activity to the original value.

At the end of the experiment, 20 g of cellulose had been hydrolysed. This was 7 times higher than when the same amount of enzyme was used in a batch reaction.

ACKNOWLEDGEMENT

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บทคัดย่อ

ในการศึกษาผลกระทบจากอัตราการไหลของ permeate ที่มีต่อการย่อยสลายเซลลูโลสโดยเอ็นไซม์เซลลูเลสจาก *Scellulophilum* ใน membrane reactor ขนาด 50 มล. โดยพิจารณาจากปริมาณน้ำตาลทั้งมด และกลูโคสที่เกิดจากปฏิกิริยาไฮโดรไลซิส ในเวลา 48 ชม. พบว่าเมื่อเวลาผ่านไป 24 ชม. กลูโคสที่เกิดขึ้นมีปริมาณ 80-90% ของน้ำตาลทั้งหมด และการปรับอัตราการไหลของ permeate ในพิสัย 15.1-26.0 มล/ชม. มีผลกระทบน้อยมากต่อปฏิกิริยาไฮโดรไลซิส นอกจากนี้พบว่าอาจมี enzyme inactivation เกิดขึ้นในปฏิกิริยาไฮโดรไลซิสระยะยาวที่ดำเนินติดต่อกัน 6 วัน

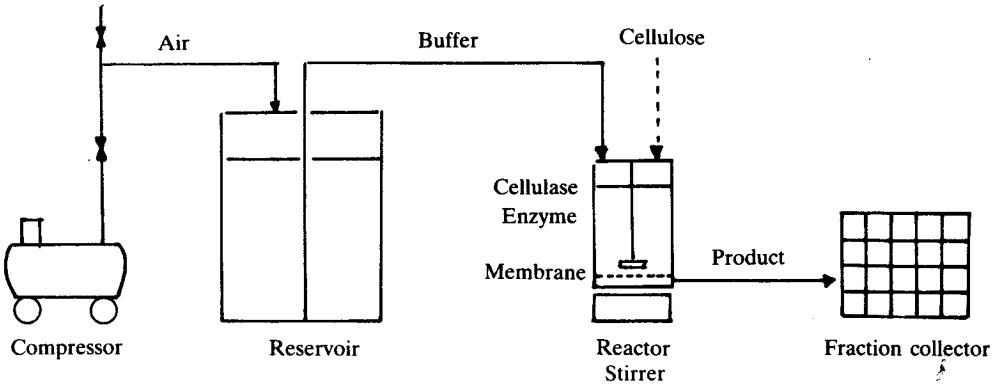

Fig. 1. Flow diagram of a continuous reactor with an ultra-filter membrane for the hydrolysis of cellulose.

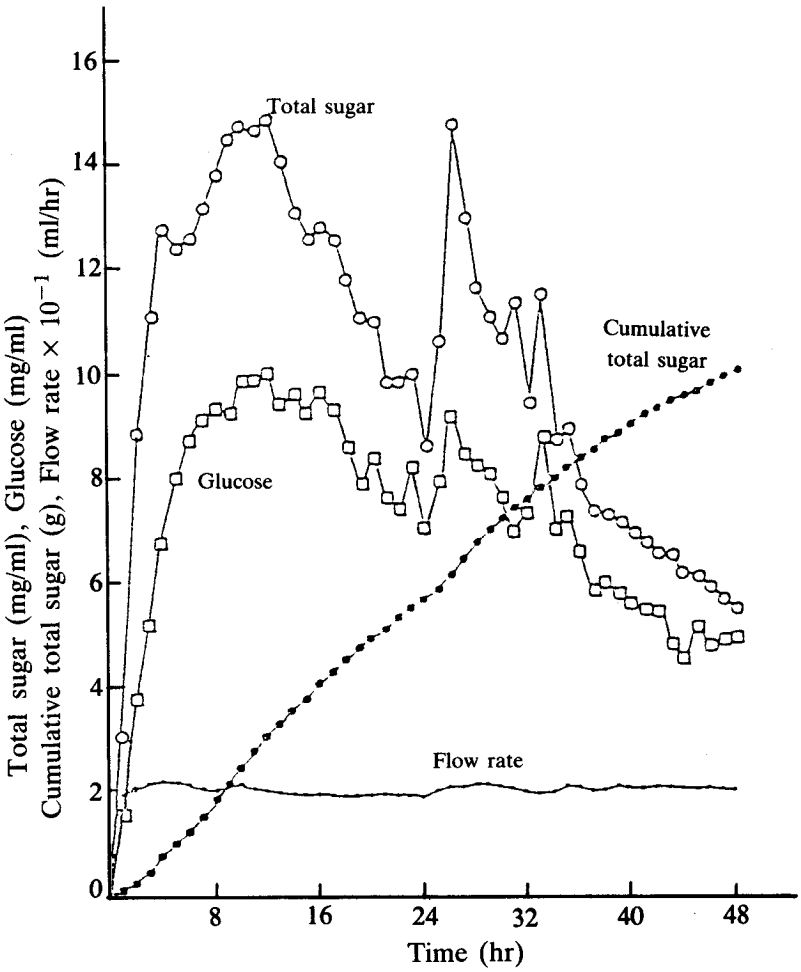

Fig. 2. Time courses of cellulose hydrolysis in an ultra-filter membrane reactor. Reaction was started with the addition of 5 g cellulose into the reactor followed by further additions of 2.5 g cellulose at 8 and 24 hr. The average permeation rate was 20.5 ml/hr (calculated after the reaction).

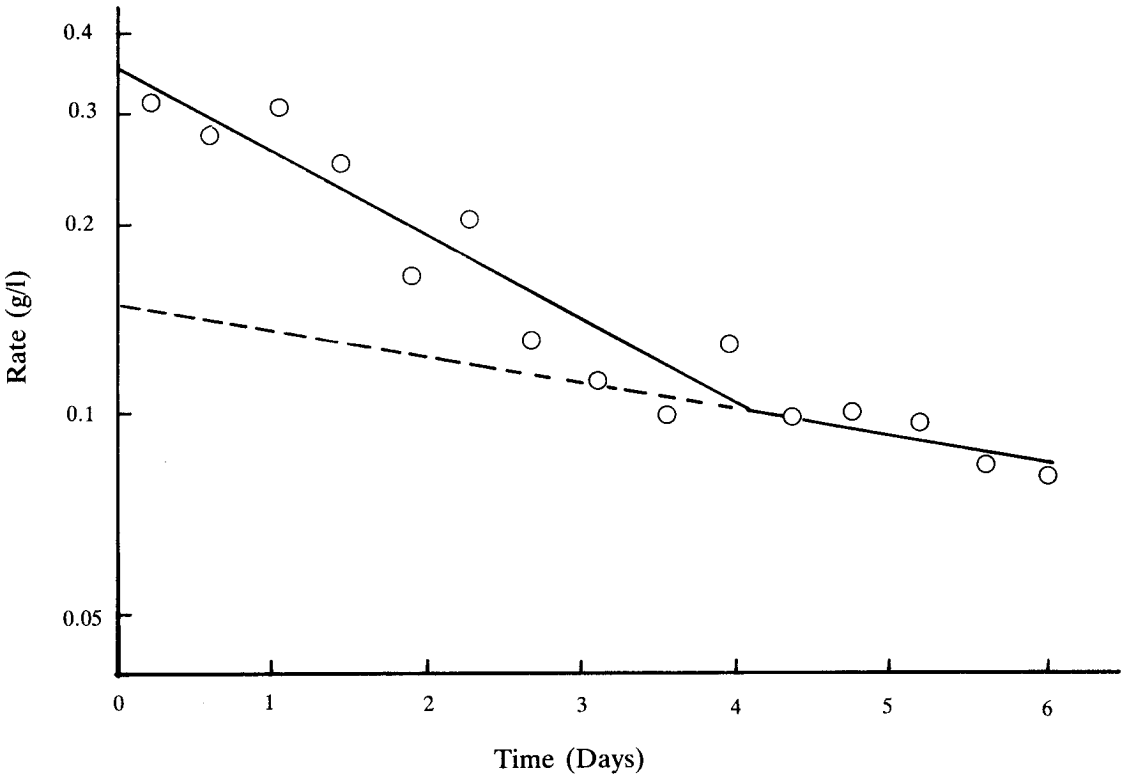

Fig. 3. Time course of enzyme inactivation in the reactor. The inactivation constants were calculated as 0.0127 and 0.044 hr^{-1} from the first and the second parts of the line, respectively.