Degradation of synthetic xylan effluent using a membrane bioreactor

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We have built a novel membrane bioreactor for the degradation of synthetic xylan effluent. The reactor contains 30 internally skinned polysulphone membranes as an immobilization matrix for xylanase, the degrading agent, and was constructed with stainless steel to withstand high temperatures, as *Thermomyces lanuginosus* SSBP xylanase has an optimum temperature of 50°C. Overall, 85.1% of the xylanase was immobilized onto the polysulphone membranes by adsorption. Preliminary results showed that the immobilized enzyme was capable of degrading the xylan effluent. Prior to contact with xylanase, there was 0.0 μg ml^{-1} xylose, 14.2 μg ml^{-1} xylobiose and 7.2 μg ml^{-1} xylotriose present in the xylan effluent. After 180 min, the xylose, xylobiose and xylotriose concentrations were 246, 103 and 91 μg ml^{-1}, respectively. Substantial increase in degradation products is promising for the development of a larger-scale bioreactor for effluent treatment.

Introduction

Interest in the membrane bioreactor (MBR) technology for wastewater treatment has grown due to increasingly stringent environmental legislation, the opportunity for water re-use and recycling it presents, and advances in and decreased costs of membrane technology. Membrane technology has attracted much attention in recent years as a new separation method for water and wastewater treatment.[1] These separation processes have been applied to treat municipal and industrial effluent. Combining membranes with biological decomposition has resulted in a new concept: the membrane bioreactor.[2] The recent development of a new generation of more productive and less expensive ultrafiltration and microfiltration membranes make this possible.[3]

The basic reactor concept is based on the separation of enzyme and products (or substrates) by a semipermeable membrane that creates a selective barrier. Permeable solutes can be separated from the reaction mixture by the action of a driving force (such as chemical potential, pressure, electric field) that is present across the membrane. Membranes can also be used in a reactor exclusively as a matrix for immobilization of the enzyme, without any separation involved.[4–7] The biocatalyst is retained within the system by the membrane, allowing the establishment of a continuous operation with substrate feed and product withdrawal.[8,9]

In the study reported here, a membrane bioreactor was constructed for the immobilization of *T. lanuginosus* SSBP xylanase to treat a synthetic xylan effluent. Appreciable quantities of xylan are present in wood extracts during pulping and pulp processing and in agricultural residues.[10] It is presently regarded as waste and often deposited in streams and rivers where it is environmentally harmful. Reducing this pollutant is desirable for improving the quality of our water supply.

Materials and methods

Strain. T. lanuginosus SSBP was isolated from soil in Durban, South Africa, identified by the CSIR and deposited in the MIRCEN Culture Collection, Bloemfontein (accession number PRI 0226).[11] The culture was grown on potato dextrose agar (PDA, Oxoid) at pH 6.5, incubated for 6 days at 50°C, and subcultured every 2–3 weeks.

Shake flask cultures. Two agar blocks (1 cm^2) with a growing 6-day-old colony of *T. lanuginosus* SSBP were used as inoculum in shake flask cultivations, which were performed in triplicate, in 250-ml Erlenmeyer flasks containing 100 ml optimal culture medium.[11] Inoculated flasks were agitated at 150 rpm on a rotary shaker for 7 days at 50°C, for maximum enzyme production. Crude enzyme present in the supernatant was extracted by filtration and used for further experiments.

β-xylanase assay. β-xylanase activity was assayed according to Bailey *et al.*,[12] using 1% birch wood xylan (Roth, Karlsruhe) in citrate buffer (0.05 M, pH 6.5) after a 5-min reaction time at 50°C. An enzyme unit (nkatal) is defined as the release of one nmol of substrate in one second.

Protein determination. Soluble protein in the supernatant was estimated by the bicinchoninic acid (BCA) method of Smith *et al.*[13] using a Pierce BCA Protein Assay Reagent Kit (Pierce, Rockford, Illinois).

HPLC analysis. A Dionex D500 HPLC coupled to an ED 40 electrochemical detector was used to analyse the xylan effluent. The system is designed mainly for carbohydrate analysis, and offers the most sensitive mode of detection available for carbohydrates. Separation arises by ion exchange. A CarboPac PA100 column was used for the analyses. The mobile phase consisted of 250 mM NaOH/degassed deionized water (1:1, volume ratio) at a flow rate of 1.0 ml min^{-1} and a run time of 35 min. Xylose, xylobiose, xylotriose and xylotetraose were used as standards.

Capillary membrane bioreactor construction. A multi-capillary membrane reactor was constructed in a shell-and-tube configuration. Thirty capillary membranes (each 540 mm long) were encased in a stainless steel shell (Fig. 2) with an outer diameter of 20 mm. The total effective membrane area was 0.061 m^2. The reactor was operated at a pressure of 100 kPa with a flow rate of 28.1 l h^{-1} and a trans-membrane flux of 163.7 l m^2 h^{-1}. The reactor was operated by recycling the substrate solution through the shell side of the capillary, and also recycling the permeate from the lumen (Fig. 1).

Immobilization of xylanase. Immobilization was performed by adsorption in the assembled bioreactor. The capillaries were initially pretreated using deionized water and then precleaned with 0.1 M NaOH for 1 h. Thereafter, a second wash was performed using deionized water. For the enzyme immobilization procedure, 100 ml crude xylanase (1.4 mg ml^{-1}) was combined with 400 ml citrate buffer (pH 6.5). The enzyme solution was then recirculated on

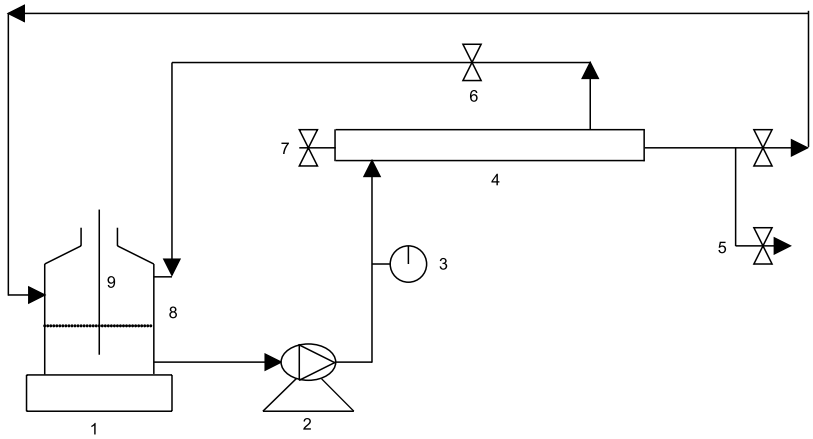

Fig 1. Schematic illustration of the bioreactor showing the mode of operation. **1**, Magnetic stirrer and heater; **2**, peristaltic pump; **3**, pressure gauge; **4**, capillary reactor (shown in detail in Fig. 2); **5**, sample port; **6**, flow regulator clamp; **7**, dead-end; **8**, vessel; **9**, thermometer.

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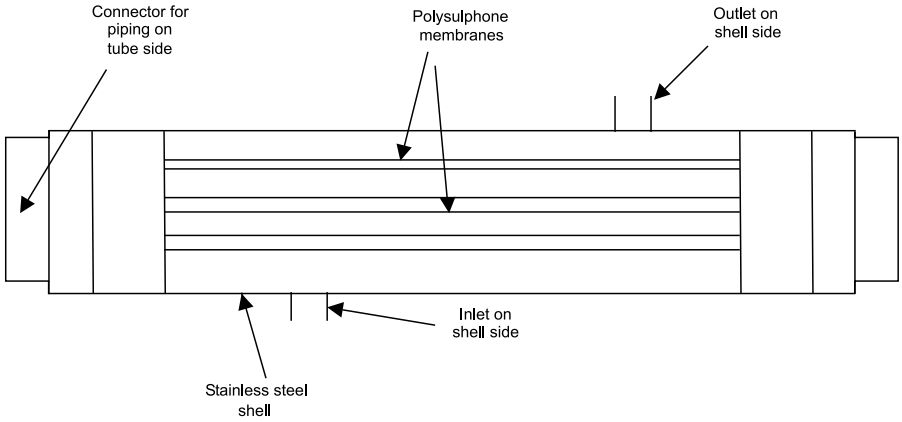

Fig. 2. Design of the capillary bioreactor.

the shell side of the reactor for 15 min before a back-pressure of 100 kPa was applied to drive the enzyme into the pores of the membrane. Samples were taken at 10-min intervals for 1 h to determine enzyme activity. The membrane was then rinsed with citrate buffer to remove any enzyme not immobilized.

Bioreactor operation. A synthetic effluent was prepared by dissolving 50 g birchwood xylan (Roth, Karlsruhe) in 0.5 M citrate buffer (pH 6.5). Prior to testing, a heating block was used to bring the temperature of the xylan effluent to 55°C. Thereafter, the reactor was operated in continuous mode with a back-pressure of 100 kPa, which allowed the substrate to pass through the membrane and make contact with the enzyme. Samples were collected at 20-min intervals for 3 h for analysis by HPLC. As a control, xylan effluent was passed through the reactor, prior to enzyme immobilization, to determine whether the system had any effect on the degradation of the effluent.

Results and discussion

Enzyme immobilization. Xylanase used in the experiments had an initial activity of 10 800 nkat ml^{-1} and a protein concentration of 0.29 mg ml^{-1}. The activities for the enzyme obtained during the immobilization period are shown in Table 1.

Xylanase activity was measured before and after immobilization, thus accounting for non-immobilized enzyme. Table 1 shows that 85.1% of the enzyme was immobilized. A small amount of enzyme activity (120 nkat ml^{-1}) was detected in the washing after the membrane was rinsed to remove any non-immobilized enzyme. Further research is necessary to determine to what extent the enzyme is inactivated when immobilized on the membrane.

Degradation of xylan effluent. The xylan effluent was heated to 55°C before the reactor pump was switched on. This had to be carried out because initial tests showed that there was a 5°C fall in temperature during the passage of the xylan effluent from the feed vessel to the capillary reactor. Lagging the temperature helped keep the reactor at the 50°C optimum for xylanase. Samples were taken at 20-min intervals and analysed for degradation products by HPLC. Xylose, xylobiose and xylotriose could be so quantified. However, xylotetraose eluted on the gradient of the chromatogram and therefore could not be quantified correctly. Results for only three sugars are therefore shown (Fig. 3).

Table 1. Xylanase activity during immobilization.

Time (min)	Activity (nkat ml^{-1})*
0	10 800 ± 116
10	7 420 ± 74
20	5 540 ± 29
30	4 080 ± 46
40	3 550 ± 30
50	2 460 ± 42
60	1 610 ± 35

*Each value represents the mean ± standard deviation of three replicates.

There was a fivefold increase in xylose concentration between 20 and 60 minutes. Twofold and threefold increases were observed for xylobiose and xylotriose, respectively, during the same period. This rapid degradation could be attributed to the favourable conditions of temperature and pH. Initial traces of xylobiose and xylotriose were evident in the effluent prior to contact with xylanase. This was, however, normal as the xylan is composed of repeating units of xylose. To verify this, an HPLC analysis of the effluent, prior to contact, revealed the presence of 14.2 μg ml^{-1} xylobiose and 7.2 μg ml^{-1} xylotriose. No xylose was detected.

This study is novel and direct comparison with related studies reported in the literature is limited. However, Boshoff *et al.*[14] and Edwards *et al.*[15,16] have shown that the enzyme, polyphenol oxidase, could be immobilized on polysulphone membranes. Their research using single-capillary membrane bioreactors proved invaluable in the development of the multi-capillary system used in our study.

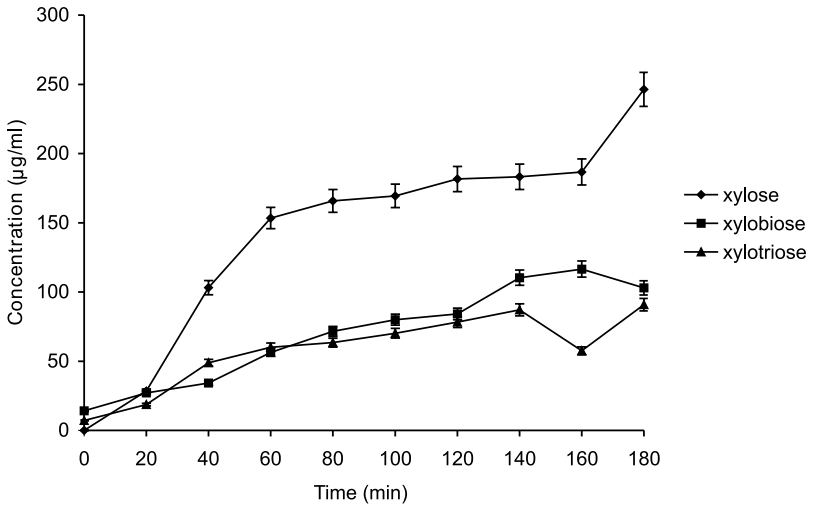

Fig. 3. Substrate conversion and product formation by immobilized xylanase. Each value is the mean ± standard deviation of three replicates.

Conclusions

These results show that xylan degraded significantly to xylose and its subunits. The immobilization of the enzyme on a membrane allows it to be re-used over time. An advantage of this process is that it is continuous, and therefore has many advantages over batch reactors, which are prone to well-documented limitations including batch-to-batch oscillations, high labour costs, frequent start-up and shut-down procedures, and the need for biocatalyst recovery after each batch.[9] Investigations to enhance the durability of the enzyme and to scale-up the reactor seem worth pursuing.

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