Studies on Commercial Aspects of Xylanase from Chainia Species

Rita Varma*, Sanjay Nene, B A Baliga
Chemical Engineering Division, National Chemical Laboratory, Pune 411008, India
and
Cynthia Elias
Biotechnology Laboratory, National Research Centre, Quebec, Canada

Received: 20 April 1999; accepted: 30 August 1999

Production of xylanase from Chainia species in a fermenter was standardized to a one litre scale. The substrate, wheat bran, was varied from 3 per cent to 5 per cent and the pH profile monitored. When 3 per cent and 4 per cent wheat bran was used the major portion of the xylan was utilized for biomass production. With 5 per cent substrate a maximum activity of 29.78 IU/ml in 72 h was achieved. The conditions were easily scaled up to 10 l. Many commercial organic nitrogen sources were studied, at shake flask level, 2 per cent soybean meal supplemented with 0.2 per cent yeast extract, gave activities comparable to when 2 per cent yeast extract was used in the media. Separation of enzyme from fermentation broth was tried by two methods, ultrafiltration and solvent precipitation. Ultrafiltration was carried out using cellulose acetate membrane cut off 5000. However, a three-fold concentration of fermentation broth gave only a little over 50 per cent of the original xylanase activity. The solvents used for precipitation of xylanase from fermentation broth were ethanol and acetone. Three volumes of ethanol gave 99 per cent xylanase recovery with a 20-fold increase in specific activity. Whereas two volumes of acetone gave 93.5 per cent recovery with 10-fold increase in specific activity. Xylanase activity in a clarified fermentation broth, stored at 10°C, retained 90 per cent of the original activity, it reduced to half its value only after three months.

Introduction

For commercial viability of a bioprocess, it is essential to have a scale up study of its production using optimal conditions and media composition. Downstream processing is a major cost effective part of any biotechnology process. A study for recovery of the product fermentation broth would be an essential requirement for commercialization of any process. Presently emphasis being on environment friendly processes, xylanase is being increasingly utilized as prebleaching agent in paper and pulp industry.1-3.

The sclerotial actinomycetes Chainia (82-5-1) secretes extracellular, cellulase free, single species xylanase4 and is a low molecular weight enzyme. Its extensive stability test has been carried out. All these features make its utilization in paper and pulp industry an attractive possibility. In this paper we report the results of the features essential for the economical feasibility of this enzyme at industrial scale. The studies include scale-up in a fermenter; a shake flask study to replace the yeast extract in media by cheaper nitrogen sources separation of product from fermentation broth and finally the stability of the enzyme.

Materials and Method

Fermenter Studies

The details of the strain maintenance and microbiological features have been reported earlier. Studies on optimal fermenter conditions were carried out in a one litre Gallenkamp fermenter. The total working volume was made up to 700ml. A 7 d-old sporulating slant was used for inoculations. The inoculum had, media composition -3 per cent wheat bran and 1 per cent yeast extract, volume 7 per cent and 48 h-old. The volume of media in the fermenter was 600ml, which had wheat bran as the carbon source (varied from 3 per cent-5 per cent) and 1 per cent yeast extract as the nitrogen source. The fermenter conditions were as follows initial pH was 6.5, 28°C, air 1-1.5 VVM, and agitation 200
rpm. A pH probe fixed in the fermenter monitored the pH, samples were removed, aseptically, at regular intervals after 24 h and enzyme activity determined. The fermentation was then scaled up to 101 in a New Brunswick Fermenter, using the same conditions.

**Commercial Organic Nitrogen Sources**

The other commercial nitrogen sources studied were cotton seed meal, soybean meal, casein and corn steep liquor. 25 per cent wheat bran was used as carbon source. A 250 ml conical flask containing 50 ml media, with the appropriate nitrogen source was inoculated with 5 ml inoculum as described for the fermenter. The flasks were kept on the rotary shaker at 200 rpm, 28°C and harvested after 96 h. The fermentation broth was clarified by centrifuging and the activities were determined.

**Downstream Processing**

**Ultrafiltration**—A cellulose acetate membrane having cut off 5000, used for the ultrafiltration studies, was cast in our laboratory of Polymer Science Division. The laboratory scale trials were carried out in an amicon ultrafiltration unit, which was scaled up to 10 l capacity in a crossflow unit.

**Solvent Precipitation**—The solvents tried were acetone and ethanol. Solvents were added to chilled centrifuged broth, ratio of broth: solvent was varied and the mixture cooled at 10°C overnight. The precipitate was collected by centrifugation and then dissolved in a small amount of 50 mM acetate buffer pH 6.0. The activity and protein concentration of the extract were determined. The activities and proteins were calculated on the basis of whole broth so as to make the results comparable.

**Enzyme Stability**

The shelf-life of the enzyme was studied by storing the clarified broth at 10°C and determining its activity at regular intervals over a period of three months until activity had reduced to nearly half the original activity.

**Assay**—The xylanase activity was estimated by incubating 0.5 ml of suitably diluted culture filtrate in 100 mM sodium phosphate buffer (pH 6.3) for 30 min. The reducing sugar formed was estimated by adding 1 ml dinitrosalicylic acid (DNSA) reagent, boiling for 5 min and taking absorbance at 540 nm. One unit of enzyme activity corresponds to one micromole of xylose produced per minute under the assay conditions.

**Results and Discussions**

**Fermenter Studies**

The pH of fermenter rose steadily throughout the experiment. The results plotted in Figure 1 indicate that pH is on the higher side when wheat bran concentration is 3 per cent, increased levels of substrate seems to have some neutralizing effect during the course of fermentation.

A study of varying substrate concentration shown in Figure 2, indicated that in the case of 3 per cent and 4 per cent wheat bran, the maximum activity is achieved after 46 h, with 4 per cent showing a slight increase. When the wheat bran was 5 per cent the activity continues to rise to 29.76 IU/ml in 72 h, after which there was slight fall, indicating depletion of substrate. Enzyme production begins only after 24 h. It was apparent that when substrate concentration was 3 per cent and 4 per cent a major part of xylan in the wheat bran was utilized for biomass production leaving very little for xylanase production. By increasing the wheat bran to 5 per cent the enzyme activity was more than doubled. A shake flask study with 6 per cent wheat bran indicated no appreciable increase in enzyme activity. Reports of other raw materials used as xylan sources, for the production of xylanase production are corn cobs, beechwood xylan, and sugar-beet pulp. A scale-up of these conditions in 10 l New Brunswick fermenter gave completely reproducible values. Thus, it is likely a further scale-up of the process will not pose any problems.

**Commercial Organic Nitrogen Sources**

Results of the different nitrogen sources used are presented in Table 1. Of all the nitrogen sources
available cheaply in bulk quantities, only 2 per cent soybean meal supplemented with 0.2 per cent yeast extract gave activities comparable to the standard 1 per cent yeast extract. One per cent soybean supplemented with 0.2 per cent yeast extract gave lower activities than just 2 per cent soybean meal, it is possible some yeast extract is essential to initiate the biomass production. However, soybean meal could successfully replace major portion of the yeast extract, which is the only expensive ingredient of the media composition. In the case of xylanase produced by the fungus *Thermomyces lanuginosus* of the different organic nitrogen sources studied soya meal was the least suitable.

As wheat bran and soybean are both insoluble, replacing yeast extract with soybean meal in a fermenter caused stirring problems, especially as *Chainia* has mycelial growth. The viscosity of the fermentation broth increases considerably, causing oxygen transfer problems. Purkarthoef er et al. observed and studied shear rate problems for production of xylanase from corn cobs by

*Thermomyces lanuginosus*.

**Downstream Processing**

**Ultrafiltration**—The fermentation broth was concentrated three-fold, after which the flux of the membrane reduced to half the initial flux. Results tabulated in Table 2, indicate an appreciable portion of the of the enzyme leaked into the permeate, also

<table>
<thead>
<tr>
<th>N source</th>
<th>Activity (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One per cent yeast extract</td>
<td>21.30</td>
</tr>
<tr>
<td>Two per cent cotton seed meal + 0.2 per cent yeast extract</td>
<td>8.61</td>
</tr>
<tr>
<td>Two per cent soybean</td>
<td>14.50</td>
</tr>
<tr>
<td>Two per cent soybean + 0.2 per cent yeast extract</td>
<td>19.66</td>
</tr>
<tr>
<td>One per cent soybean + 0.2 per cent yeast extract</td>
<td>11.80</td>
</tr>
<tr>
<td>Two per cent corn steep liquor</td>
<td>9.60</td>
</tr>
<tr>
<td>One per cent casein</td>
<td>10.63</td>
</tr>
</tbody>
</table>
Table 2—Concentration of xylanase in the fermentation broth by ultrafiltration

<table>
<thead>
<tr>
<th>Broth</th>
<th>Volume (ml)</th>
<th>Activity (IU/ml)</th>
<th>Total activity (IU)</th>
<th>Per cent activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>8000</td>
<td>30.24</td>
<td>241920</td>
<td>100.00</td>
</tr>
<tr>
<td>Retentate</td>
<td>2800</td>
<td>34.65</td>
<td>132208</td>
<td>54.65</td>
</tr>
<tr>
<td>Permeate</td>
<td>5200</td>
<td>3.80</td>
<td>19760</td>
<td>8.16</td>
</tr>
</tbody>
</table>

Table 3—Separation of xylanase from fermentation broth by solvent precipitation

<table>
<thead>
<tr>
<th>Solvent:Broth ratio</th>
<th>Initial Activity (IU/ml)</th>
<th>Extracted Activity (IU/ml)</th>
<th>Specific activity (mg/ml)</th>
<th>Per cent enzyme Extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone:broth 1:1</td>
<td>26.77</td>
<td>nil</td>
<td>3.50</td>
<td>nil</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>30.10</td>
<td>28.15</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td>3:1</td>
<td>28.30</td>
<td>24.80</td>
<td>3.50</td>
</tr>
<tr>
<td>Ethanol:broth 1:1</td>
<td>26.77</td>
<td>nil</td>
<td>3.50</td>
<td>nil</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>30.10</td>
<td>23.62</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td>3:1</td>
<td>28.30</td>
<td>28.00</td>
<td>3.50</td>
</tr>
</tbody>
</table>

There are some losses due to denaturing of enzyme and some adsorption on the membrane. The total recovery with our cellulose acetate membrane was just a little over 50 per cent.

Solvent Precipitation—The results of solvent precipitation are presented in Table 3. When equal volumes of solvent and broth are used no xylanase is extracted, other proteins are first precipitated in the case of both solvents. In the case of acetone a ratio of 2:1, 93.5 per cent of the xylanase is extracted with ten-fold increase in specific activity. An increase in solvent results in the precipitation of other proteins. Whereas, three volumes of ethanol added to broth gave best results, 99 per cent xylanase is recovered with a 16-fold increase in specific activity.

Enzyme Stability

The stability of the xylanase is shown in Figure 3. It is evident that clarified broth retains 90 per cent of its activity for a month, it reaches half its activity only after a period of three months. The results indicate that xylanase from Chainia sp. has good stability for its commercial exploitation. A

Figure 3—Stability of xylanase in fermentation broth over a period of three months.
30 per cent reduction of enzyme activity after several months storage at -20°C in the case of T.lanuginosus and H.lanuginosus have been observed.

**Conclusions**

As xylanase from *Chainia* is a low molecular, cellulase free and single species xylanase, it has potential use in paper and pulp industry. Results show that scaling-up of the fermenter gives reproducible results. Wheat bran and yeast extract are the only two ingredients of the media. If fermentation is carried out using a more powerful stirring device or an airlift fermenter soybean meal could be used as cheap nitrogen, the cost of chemicals could be drastically reduced. With a variety of ultrafiltration membranes constantly hitting the market, a more efficient recovery of the product using membrane technology should be possible. However, since precipitation using ethanol as solvent gives complete recovery of xylanase in a simple manner it can easily be adapted at commercial scale. If preferred, acetone can also be used as an alternate solvent. Both solvents are cheap and can be recovered for reuse. The stability of xylanase activity in the whole broth for period of one month, at 10°C indicates immediate downstream processing is not essential. All these features make xylanase from *Chainia* sp. a promising enzyme for industrial production.

**Acknowledgments**

Authors are grateful to Department of Biotechnology, New Delhi for providing financial assistance. The authors thank NCIM (National Collection of Industrial Microorganism), NCL, for supplying the culture and Dr S S Kulkarni of Polymer Science Division, NCL, for the ultrafiltration membrane.

**References**