2.76 Production and Separation of an Endo-polygalacturonase from *Kluyveromyces marxianus* Fermentation

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Keywords: fermentation, aqueous two-phase extraction, endo-polygalacturonase, *Kluyveromyces marxianus*

Kluyveromyces marxianus fermentation in the production of pectinase has several attractive characteristics, mainly associated with the low secretion of undesirable proteins to the fermentation broth. Ion-exchange chromatography and gel filtration chromatography showed that about 40% to 50% of total protein in the clarified fermentation broth consisted of endo-polygalacturonase (endo-PG). As consequence downstream processing of a fermentation broth to produce a crude, stable enzyme product, free of proteolytic enzymes, may also be simplified. As aqueous two-phase extraction is an useful and scalable separation method for enzymes and other biological substances, it is of interest to develop this technique for endo-PG purification and concentration.

Since molasses cane is, probably, one of the cheapest sucrose sources available from industry, batch fermentations for yeast selection among 8 *Kluyveromyces marxianus* strains were done using a synthetic medium containing sucrose as carbon source at an initial concentration of 10 g/L. Experiments were done in flasks at initial pH 5, 30 °C and 150 rpm. For all strains the enzyme production was growth related. Total substrate consumption and maximum activity were reached at 16 hours of fermentation. Strain CH0-1 was found to be the best endo-PG producer. For this strain additional fermentation experiments were performed using molasses cane (sucrose was about 10 g/L) as carbon source confirming previous results.

The random copolymer of ethylene oxide and propylene oxide (Ucon 50-HB-5100, from Union Carbide, USA) was selected as phase-forming polymer. A new aqueous two-phase system based on this polymer and $(NH_4)_2SO_4$ was developed. The partitioning of endo-PG and total protein from a clarified fermentation broth in Ucon-$(NH_4)_2SO_4$ ATPS is investigated It was found that both endo-PG and total protein strongly partitioned to the bottom salt-rich phase. However, partitioning difference exists between enzyme and total protein, which still implies the possibility of enzyme purification. A separation scheme with polymer recycling was proposed to separate the endo-PG for commercial use. After two steps of two aqueous two-phase extraction in 27.6%Ucon-3.2%$(NH_4)_2SO_4$ systems, an enzyme recovery up to 90%, a concentration factor of more than 5 and a purification factor of 1.8 could be achieved (the maximum purification is 2.0 to 2.5 for any separation method, since 40% to 50% of total protein consists of endo-PG). 80-90% of the total polymer added in the systems could be recovered for polymer recycling from the top Ucon-rich phase by raising temperature up to 80°C, with no reduction in purification factor.

The present work revealed the possibility of economic preparation of purified and concentrated endo-PG from molasses cane for food industry.