Urokinase production by animal cell culture in hollow fiber bioreactor

by

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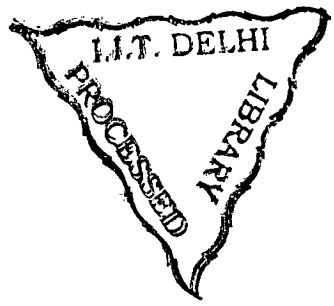
1. Urokinase production



CERTIFICATE

This is to certify that the thesis entitled "Urokinase production by animal cell culture in hollow fiber bioreactor", being submitted by **Ms Shilpa Sharad Khaparde** to the Indian Institute of Technology, Delhi, for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The research reports and results presented in the thesis have not been submitted to any other university or institute for the award of any other degree or diploma.

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Dedicated to

My pillars of strength

Shrimati. Shubhada S. Chapadgaonkar &

Mrs. Swati S. Khaparde

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ABSTRACT

The present invention reveals a novel process for production of urokinase by mammalian cell culture. The production process has been specifically designed to reduce the operational costs that are contributed by expensive culture medium, losses due to multiple-step processing and proteolytic degradation. This has been achieved by employing high density culture in hollow fiber bioreactor (HFBR), development of specialized medium, manipulating the bioreactor process variables to maximize the productivity and minimize the cost. The most attractive feature of the technology is simultaneous capture of urokinase produced in the HFBR by affinity purification column integrated to the bioreactor. This enables production and purification of urokinase as a single automated operation.

Adherent human kidney cell line (HT-1080) was used for urokinase production. The basal medium (DMEM/F12 1:1) enriched with 10% v/v new born calf serum (NBCS) was employed for rapid proliferation of adherent cells. However, in the production phase, serum concentration could be successfully reduced to 1% v/v resulting in increase in urokinase production with no adverse effect on cell viability. Since the concentration of serum in the urokinase production medium was minimal, the downstream processing was immensely simplified and the simultaneous recovery of urokinase directly from the HFBR was made possible.

The novel strategy applied for medium optimization was based on the supplementation of amino acids that occur most abundantly in the primary structure of urokinase. The amino acid requirement of the HT-1080 cells in the production phase was identified by HPLC analysis of the culture broth. The optimized production medium

consisted of basal medium enriched with enhanced concentration of aspartic acid, glycine, serine and arginine that were found to be consumed rapidly during urokinase production phase. The medium optimization resulted in approximately about 240% increase in urokinase production. This was partly due to higher cell viability obtained in the optimized low serum medium. The effect of temperature shock on HT-1080 cell metabolism and urokinase production was also studied. Urokinase production was enhanced by lowering culture temperature to 34 °C. Moreover higher prourokinase fraction could be recovered by lower culture temperature.

The strategy for urokinase production in hollow fiber bioreactor was developed and the process variables like the medium feed, recirculation and harvest rates were optimized. In the optimized HFBR system, urokinase production of the order of 4.5×10^5 PU/day could be obtained continuously for more than a month. The concentrated product stream and low protein load rendered HFBR system suitable for integration of production and purification process. Purification of urokinase by affinity chromatography using p-amino benzamidine Sepharose, an analog of urokinase inhibitor, has previously been standardized in our laboratory (Bansal, 2005). Since the p-amino benzamidine-Sepharose matrix was found to be stable and non-toxic to the cells, the culture broth could be directly loaded on to the affinity chromatographic column making it an ideal material for integrating with the HFBR. The integration has unique advantages including, feed back inhibition-free environment, reduction of loss of urokinase activity, improvement of process economy by reduction of processing steps and decrease in process downtime. The integrated process resulted in the production of approximately about 4.3×10^6 PU/day of urokinase having specific activity of 86,198 PU per mg. The capture of

urokinase by the p-amino benzamidine Sepharose column eliminates feed back inhibition and enhances productivity of the cells resulting in about 10-fold increase in urokinase production as compared to conventional process wherein urokinase is recovered by offline separation techniques.

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