

PRODUCTION OF UROKINASE IN HOLLOW FIBER BIOREACTOR BY HUMAN KIDNEY CELL LINE

Biotech Consortium India Limited (BCIL) is seeking companies interested in commercializing a technology used for the production of urokinase in hollow fiber bioreactor by human kidney cell line. This method has been developed at Indian Institute of Technology, New Delhi, India. It enables the production of highly concentrated urokinase continuously for prolonged time duration.

Introduction:

Urokinase, a serine protease, catalyzes the conversion of plasminogen to plasmin, which is responsible for dissolving the clots of fibrin in blood vessels, thus making urokinase an enzyme of great clinical importance. Urokinase has been widely used as an effective thrombolytic agent for medication of deep venous thrombosis, acute myocardial infarction, pulmonary embolism and peripheral arterial occlusion. Urokinase is less allergic and is not antigenic. Even though streptokinase is less expensive for treating patients suffering from thrombosis, urokinase is preferred as cost effective treatment, due to the drawback of streptokinase having higher incidence of allergic reactions.

Commercial relevance of the product/process:

Thrombosis has proven to be a major cause of death in the modern world. Therefore the demand of clot busting drugs has risen tremendously over the past years. Urokinase (UK; E.C. 3.4.99.26) has been widely used as an effective thrombolytic agent for medication of acute myocardial infarction and peripheral arterial occlusion. Scarce supplies and inflation of drug prices have forced the physicians to resort to alternative drugs. The method developed indigenously at Department of Biochemical Engineering & Biotechnology, IIT, New Delhi, enables production of highly concentrated urokinase continuously for a prolonged duration of time. The urokinase concentration obtained is approximately **10 times concentrated** as compared to what is available in literature. Consequently it would result in *volumetric scale down of purification process*, improving the overall economy. High urokinase levels are maintained for prolonged duration (more than one month) once the culture is established in the hollow fiber bioreactor.

Scale at which the technology has been developed:

Hollow fiber cartridge employed for urokinase production supports the growth of about 5×10^9 cells and harvest of 10,000 PU per ml per day and about 4.5×10^5 PU/day (30 doses) of urokinase.



PRODUCTION OF UROKINASE IN HOLLOW FIBER BIOREACTOR BY HUMAN KIDNEY CELL LINE

Salient features of the technology:

1. Human kidney cell line HT-1080 was used for urokinase production.
2. A low serum specialized medium was developed for urokinase production. The culture duration could be conveniently divided into two phases growth phase and production phase. Lowering the serum concentration and supplementing the basal medium with amino acids induced a shift in metabolism towards high urokinase production while maintaining viability of the cells.
3. The cells were inoculated in a hollow fiber bioreactor (molecular weight cut off 5 kDa) and allowed to grow for first 6 days and then the metabolic shift was induced and extra capillary space was flushed frequently to harvest urokinase. The culture maintained the urokinase productivity for over 30 days.

Patents & Publications:

Indian patent filed for the Production of urokinase in hollow fiber bioreactor by human kidney cell line (Application No.1759/Del/2006 August 1).

Roychoudhury PK, Gomes J, Bhattacharyay SK, and Abdulah N: Production of urokinase by HT 1080 human kidney cell line. *Artif Cells Blood Substit Immobil Biotechnol* 1999, Sep-Nov;27(5-6):399-402.

Roychoudhury PK, Khaparde SS, Mattiasson B and Kumar A: Synthesis, regulation and production of urokinase using mammalian cell culture: a comprehensive review. *Biotechnology Advances*.2006 Sep-Oct;24(5):514-28. Epub 2006 May 26.

Kumar A, Bansal V, Andersson J, Roychoudhury PK and Mattiasson B: Supermacroporous cryogel matrix for integrated protein isolation. Immobilized metal affinity chromatographic purification of urokinase from cell culture broth of a human kidney cell line. *Journal of Chromatography A*. 2006 Jan 20;1103(1):35-42. Epub 2005 Dec 20.

Kumar A, Bansal V, Nandakumar KS, Galaev IY, Roychoudhury PK, Holmdahl R, and Mattiasson B: Integrated bioprocess for the production and isolation of urokinase from animal cell culture using supermacroporous cryogel matrices. *Biotechnology and Bioengineering*. 2006 Mar 5;93(4):636-46.



PRODUCTION OF UROKINASE IN HOLLOW FIBER BIOREACTOR BY HUMAN KIDNEY CELL LINE

Advantages of the proposed technology over the existing options:

In the past, the only source of urokinase has been human urine. However, isolation of this enzyme from urine entails the near insurmountable quantities of human urine, because yields are very small. The present technique utilizes the cultured human kidney cells for the production of urokinase leading to better quality and consistency of the source material.

Detailed analysis of spent culture medium has provided valuable information on nutrient limitation or toxic metabolite accumulation. The urokinase production medium was specifically designed to avoid limitation and toxicity due to inhibitory metabolites and maximise the production leading to about 4 times increase in urokinase production over original titers.

Authors in the past have generally used the microcarrier culture technology for urokinase production. However in microcarrier culture of kidney cells the cell concentration is low, leading to low productivity and product concentration. Moreover, experimental studies at IIT indicate that the urokinase production as well as cell viability is affected due to shear stress generated in stirred tank reactor used for microcarrier culture. Conversely, hollow fiber bioreactors (HFBR) offer several significant advantages over the microcarrier culture. Hollow fiber reactors closely resemble the *in vivo* conditions and offer several significant improvements in reactor performance. The cells experience minimal shear forces in the extracapillary space and therefore can be grown at very high densities (10^9 cells/ml). The cells immobilized on hollow fibers are also much easily adaptable to low serum concentrations. Moreover the molecular weight cut of the hollow fiber membranes was selected in such a way that urokinase was retained in the extracapillary space enabling the harvest of a highly concentrated product.

About BCIL:

BCIL was incorporated as public limited company in 1990 under the Indian Companies Act 1956. It is promoted by the Department of Biotechnology, Government of India and is financed by several all India financial institutions, venture capital funds and the corporate sector. BCIL has been actively involved in technology transfer, project consultancy, fund syndication, information dissemination, and manpower training & placement related to biotechnology over the last decade and half. BCIL has transferred more than 15 technologies in the last 5 years using its expertise in facilitating licensing agreements that allow healthy and productive cooperation between the inventor and the licensee.
