中国化学工程学报 1999 7 (2): 139-144 ISSN: 1004-616x CN: 44-1063/R

Volume 7

Simultaneous Cell Disruption and Aqueous Two-Phase Extraction for Isolation of Intracellular Recombinant Proteins

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收稿日期 1998-5-13 修回日期 网络版发布日期 接受日期 1998-10-8

摘要 A new technique was developed for the integrated processing of cell disruption and aqueous two-phase extraction in a high-speed bead mill to separate intracellular proteins from microbial cells. The process was named as simultaneous cell disruption and aqueous two-phase extraction (SDATE). Advantages, such as, high cell disruption efficiency, biochemical activities preserved of the proteins, cell debris elimination, and prelimiary purification of the target protein were being claimed. When this technique was employed in isolating recombinant Tumor Necrosis Factor (rhTNF) from E.coli, overall protein concentration and TNF activity were found to have been increased. More than 95% of TNF was partitioned into the top phase and all the cell debris were in the bottom phase. The partition coefficient was greater than 3 and the TNF purification factor was greater than 6. It is shown that less separation steps were being utilized in the new technique, meaning a reduction in separation time and less process extractors required.

关键词 SDATE Release of intracellular proteins high speed bead mill aqueous two-phase extraction tumor necrosis factor

分类号

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Received 1998-5-13 Revised Online Accepted 1998-10-8

Abstract A new technique was developed for the integrated processing of cell disruption and aqueous two-phase extraction in a high-speed bead mill to separate intracellular proteins from microbial cells. The process was named as simultaneous cell disruption and aqueous two-phase extraction (SDATE). Advantages, such as, high cell disruption efficiency, biochemical activities preserved of the proteins, cell debris elimination, and prelimiary purification of the target protein were being claimed. When this technique was employed in isolating recombinant Tumor Necrosis Factor (rhTNF) from E.coli, overall protein concentration and TNF activity were found to have been increased. More than 95% of TNF was partitioned into the top phase and all the cell debris were in the bottom phase. The partition coefficient was greater than 3 and the TNF purification factor was greater than 6. It is shown that less separation steps were being utilized in the new technique, meaning a reduction in separation time and less process extractors required.

Key words SDATE; Release of intracellular proteins; high speed bead mill; aqueous two-phase extraction; tumor necrosis factor

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